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FUNCTIONAL ECOLOGY OF MICROBIAL FRESHWATER COMMUNITIES FROM BYERS PENINSULA (LIVINGSTON ISLAND, ANTARCTICA)

Ecología funcional de las comunidades microbianas de los sistemas acuáticos epicontinentales de la Península Byers (Isla Livingston, Antártida)

Tesis Doctoral

Carlos Rochera Cordellat

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Fdo. Carlos Rochera Cordellat

Tesis dirigida por el Dr. **Antonio Camacho González**, Profesor Titular del Departamento de Microbiología y Ecología de la Universidad de Valencia.

Fdo. Prof. Dr. Antonio Camacho González

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Abstract

This thesis studies the microbial communities inhabiting freshwater ecosystems of Byers Peninsula (Livingston Island, Antarctica). This is an ice-free area of around 60 km^2 that holds numerous lakes, ponds, rivers and wetlands. As a deglaciated region, the functioning of aquatic ecosystems is very closely linked with the surrounding land. So, when snow melting occurs during summer, interactions with the catchment become more intense, and coincide with enhanced biological activity. We conducted a systematic survey in the most representative lakes during some consecutive austral summers between 2002 and 2006. Most of these lakes are located on a plateau, where the landform facilitates water retention and they show well-defined surface outlets. According to their nutrient concentrations and the phytoplankton biomass, these lakes range merely from ultra-oligotrophic to oligotrophic. Our surveys have revealed the existence of a large standing stock of mosses (*Drepanocladus longifolius*) at the bottom of some of them, thus suggesting that primary production could mainly be allocated in the benthic compartment. On the other hand, the shallow lagoons located in coastal areas usually show bigger catchments, which are largely covered by mosses cushions and plants. These coastal lagoons have a higher nutrient content due to the inputs of elephant seals (*Mirounga leonina*) dwelling in their vicinities, and somewhat greater salinity due to more exposure to sea spray. Bacterial abundances vary broadly among lakes (0.5 to 6.5 x 10^6 cell mL^{-1}), thus also underlying this inland-coastal gradient of productivity. Still, these bacterial abundances are greater than is expected for these nutrient-poor lakes. We also performed a multivariate analysis that reveals other factors such as the lakes' morphometry to explain the more subtle differences in their trophic status.

In general, all the lakes hold a few species and apparently exhibit a simple food web dominated by microbial communities. The only relevant metazoan species we found are the copepod *Boeckella poppei* and the fairy shrimp *Branchinecta gaini*. Besides, the former is the only species to present a significant role in the pelagic part. Our surveys describe microbial communities dominated by small flagellates species (both colourless and plastidic forms), amoebae and a few ciliates species. These last species are mainly composed of the euplanktonic ciliate *Balanion planctonicum*, with densities in Lake Limnopolar of up to $2.8x10^3$ ind L^{-1}, just after the ice cover retreat. Otherwise, phytoplankton is composed mainly of diatoms, chrysophytes, picocyanobacteria and chlorophytes. However, these assemblages may be incidental since some species originate from the catchment or are resuspended from the benthos when turbulence in the lake increases.

One of our aims was to test the basic principles of the functional ecology in these simple environments. These lakes support plankton in truncated pelagic food webs, where the copepod *B.poppei* is the capstone predator. A strong trophic connection exists, however, between copepods and the microbial loop. We present a conceptual model of the ecological functioning of one of these lakes, Lake Limnopolar, located on the plateau, which we have adopted as a model study lake. By means of manipulative experiments, we demonstrate the potential existence of a trophic cascade in its pelagic food web. It is mediated by a strong top-down regulation of protozoa populations by copepods, which indirectly benefits pico-sized organisms (both autotrophic and heterotrophic). The experiments showed that these trophic pathways are mainly channelized through ciliates, whose abundance declined markedly in response to increased copepod densities. The selective grazing of protists over picoplankters furthermore favours a shift in the bacterial size structure. Our observations also advocate the incorporation into this food web modelling of an effective recycling of nutrients driven by these copepods. In this sense, we conjecture that a translocation of nutrients from lake's bottom to surface waters could be mediated by the copepods' diel vertical migration.

The analysis of the isotopic carbon fractionation occurring in Lake Limnopolar indicated that *B.poppei* profits mainly from pelagic resources. Besides, the liquid chromatography (HPLC) analysis of taxa-specific carotenoids revealed the ability of this copepod to produce a redistribution of the relative abundances of pelagic algal groups because of a differential grazing pressure. For instance, this indicates that chlorophytes (e.g. *Ankistrodesmus antarcticus*) , but not diatoms and chrysophytes, are favoured when copepods abound. In some periods however, we found that copepods mainly retreated to deep layers, suggesting nektobenthic behaviour. Here, we agree with those observations indicating a certain phenotypic plasticity of this species, in such a way that its feeding mode may vary depending on environmental conditions. In contrast, isotopic fractionation clearly revealed that *Branchinecta gaini* exploits benthic resources, thus demonstrating a niche segregation between the two dominant metazoans in the lake.

We demonstrate that ice dynamics is very much subject to variations due to the year-to-year meteorological variability of the region. We observed, for instance, inter-summer differences in the ice-out timing of 55 or 25 days. We demonstrate that this local variability could be an important factor to explain the limnological dynamics. Therefore, further attention has to be paid to the fact that these year-to-year variations may be large and may thus complicate the explanation of long-term variations attributed to climate change, unless we better understand such dynamics.

We partially studied the gains and losses of heat in the lake, and observed differences either with or without a frozen cover. During the ice-free periods, if solar radiation is low, the lake loses sensible heat by the shear produced by wind, although this occurs with some delay. On the other hand, density-driven currents dominate when the lake is to be ice-covered. Seasonal changes in the phytoplankton community structure occur in Lake Limnopolar in relation to ice dynamics. When the lake is ice-covered, light availability is greatly limited and nutrients diffusion is restricted because of the high water column stability. During this period, autotrophic picoplankters, both picocyanobacteria and picoprasinophytes, which usually play a minor role, account for up to around 50% of the total phytoplankton biomass at sub-surface layers. By contrast, greater abundances of larger phytoplankton (chrysophytes and diatoms) and bacteria take place with the onset of ice melting and coincide with increases in nutrients, turbulence and light availability. In other respects, and along the trophic gradient previously defined among the lakes, the relative role of autotrophic picoplankters increases as nutrient content decreases.

In general, pelagic primary production is very low in the lakes from Byers; however, the plankton's heterotrophic component is relatively abundant for such low productive rates. In relation to this, our studies suggest that the benthic communities which flourish in the surrounding areas of the lakes (principally microbial mats) could be a source of allochthonous inputs of organic carbon, as the isotopic fractionation also suggests, thus contributing to fuel bacterial production. This organic carbon may also originate from the benthic mosses growing within the lake. In this sense, an in-depth study into this subject is necessary because, if bacteria are mainly subsidised by allochthonous carbon, the idea that low-productive systems tend to be net heterotrophic is reinforced. The dissolved organic carbon (DOC) concentrations in the oligotrophic lakes of Byers Peninsula were around 1 mg L^{-1}, which seems high enough to sustain bacterial growth. The results indicate that bacteria may compete with phytoplankton for mineral nutrients, which hypothetically may occur if nutrients availability is notably scarce.

We also examined the taxonomic and physiological diversity of the benthic microbial communities at the site. For this purpose, we conducted a multi-approach study of three representative microbial mats at the site. Two of the mats (soil and pond mats) were located on the central plateau, whereas the third one (stream mat) was located in the coastal area of the South Beaches on the edge of streams. The microscopic and pigment analyses revealed that the stream mat was dominated by diatoms (genera *Navicula*, *Fragilaria*, *Stauroneis*, *Nitzschia*, *Gomphonema* and *Pinnularia*), whereas the mats' characteristics of the plateau (soil and pond mats)

were dominated by cyanobacteria (*Leptolyngbya* sp., *Oscillatoria* spp., *Phormidium* spp., *Porphyrosiphon* sp., *Nostoc* sp.). The photosynthetic activity of the three mats is comparable to that observed in similar Antarctic communities, yet relies on the local factors where they grow. The soil and pond mats were distributed over moist soils and at the bottom of ponds. Unlike the stream mat, these two mats exhibited the sheath pigment scytonemin, a higher content of exopolymeric substances (EPS) and some elemental disequilibrium (C:N:P), thus suggesting great environmental stress. The areal carbon uptake (mainly through oxygenic photosynthesis) in the three mats ranged from 2.7 to 4.2 μg C cm^{-2} h^{-1}, being these higher in the stream mat. The profiles with microelectrodes showed maximum photosynthetic activity at the sub-surface layers, which moreover revealed a more balanced stoichiometry than at the surface layers. Nitrogen uptakes also varied among mats. N_2 fixation only occurred in the mats from the plateau, and was notably higher in the soil mats. In contrast, the areal assimilation rates of combined forms (nitrate and ammonium) were higher in the stream mat.

The other benthic communities we studied are the different phototrophic biofilms which flourish in streams. Our study particularly focused on several biofilms revealing a restricted distribution within a waterfall that formed a canyon downstream. We observed up to five different communities there, whose position responded to the selective stresses exerted by stream flow and moisture. In our opinion, there is a trade-off between water current (i.e., water renewal) and nutrient availability to explain the biofilms distribution. Accordingly, the exopolymeric substances (EPS) content, stoichiometry (C:N:P) and pigment composition of the biofilms demonstrated a distinct nutritional status. Occurrence of functionally competent biofilms of chlorophytes (*Ulothrix* sp.) was restricted to the central stream channel, which likely indicates adaptation to faster flow events. The communities dominated by cyanobacteria (*Oscillatoria* spp., *Phormidium* cf. *autumnale*, *Leptolyngbya* sp.) were, in contrast, more diverse and appeared in a wide range of environmental conditions. The dominant diatoms in these biofilms were *Fragilaria capucina* s.l., *Nitzschia* cf. *gracilis*, *Nitzschia inconspicua*, *Chamaepinnularia gerlachei*, *Planothidium delicatulum* y *Gomphonema* sp., being the later related with biofilms subjected to higher drought stress. The areal photosynthetic rates (mainly oxygenic) in the biofilms ranged from 0.7 to 3.4 μg C cm^{-2} h^{-1}. Greater activities were comparable to those observed in other maritime Antarctic locations, whereas the lower values obtained in the more stressed biofilms fall in the same range observed for the cyanobacterial communities of the continental region.

We also assessed if the competitive interactions based on resource utilisation could explain the structure of the photosynthetic community in the microbial mats. Thus, a fully factorial nitrate and phosphorus addition experiment was conducted with the stream mat, which revealed that phosphorus fertilisation favours the growth of non-heterocystous cyanobacteria in relation to diatoms. Our experimental handling did not generate a detectable accretion of mat; however, the balanced nutrients availability improved phototrophic activity. As far as we know, this is the first attempt to study the effects of inorganic nutrient additions on the structure and function of an Antarctic microbial mat.

Our findings demonstrate that biotic interactions may play a key role in structuring these aquatic food webs despite strong physical control, at least when physical stressors are temporarily relaxed. This idea contrasts partly with the general contention that ecosystems in extreme environments lack biotic control. We have certainly observed that ice dynamics and water column stability greatly explain plankton succession; nevertheless, community size structure and the occurrence of metazoan zooplankton may also explain plankton dynamics. As regards microbial mats, our enrichment experiment with stream mats also suggests that a shift in regional nutrients dynamics might alter the metabolic equilibrium of these microbial communities. The freshwater ecosystems at this site are not affected by direct anthropic stressors, but are disturbed by climate or natural eutrophication processes. Accordingly, they can be effective sentinels for climate change as they are highly sensitive and integrate information about the processes occurring in the catchment. Monitoring and experimental activities should then continue in Byers to conduct an in-depth study into the consequences of climate change on both local and global scales. The knowledge acquired by this limnological study furthermore stresses the idea of establishing this Antarctic location as an international reference site for research into polar regions.

Resumen

La presente tesis se encuadra en el estudio de las comunidades microbianas que habitan los sistemas acuáticos epicontinentales de la Península Byers. Esta región constituye un área deshelada con una extensión de aproximadamente 60 km^2 situada al oeste de la Isla Livingston (Antártida). Buena parte de la Península Byers presenta una topografía suavizada, con un sistema fluvial bien desarrollado, el cual alberga numerosos lagos de diferente tamaño y cursos de agua de distinto orden. Muchos de estos lagos muestran un complejo sistema de drenaje y límites de cuencas todavía no bien definidos. La región se caracteriza por presentar altos niveles de precipitación en comparación a otras zonas del continente antárctico, las cuales se concentran principalmente en la época estival, con tasas estimadas que pueden alcanzar valores de 700 mm $año^{-1}$. Generalmente, las temperaturas estivales oscilan en torno a 0-3 ºC, mientras que las invernales muestran en promedio valores de -10 ºC, con mínimas ocasionalmente algo inferiores a -20 ºC. Este régimen térmico permite que se produzca en la zona un importante deshielo durante el verano austral. Debido a la ausencia de hielo permanente, estos sistemas acuáticos interaccionan de forma notable con la cuenca circundante. El aumento de las temperaturas puede provocar también la casi completa desaparición de la nieve, lo que coincide además con el aumento de la actividad biológica. Durante un periodo casi continuado entre los años 2002 a 2006 se han realizado durante los veranos australes distintas campañas limnológicas, estando estas centradas en el estudio de los lagos más representativos de la zona. La mayoría de estos lagos se encuentran situados en el altiplano de la península, donde la geomorfología del terreno facilita la retención de agua. En base a su concentración de nutrientes y biomasa algal, buena parte de ellos presentan un estado trófico que varía entre ultra-oligotrófico y oligotrófico. Durante las campañas llevadas a cabo, se constató la existencia en algunos de ellos de densas poblaciones de musgos bentónicos (*Drepanocladus longifolius*), lo que sugiere que una parte importante de la producción primaria podría estar emplazada en este compartimento. En contraposición a esto, las lagunas someras situadas en la zona costera poseen cuencas de captación por lo general más extensas, con una mayor cobertura vegetal en sus alrededores. Estas lagunas costeras mostraron un mayor contenido de nutrientes, debido principalmente a los aportes provenientes de las colonias de elefantes marinos (*Mirounga leonina*), así como un contenido de sales algo más elevado como consecuencia de una mayor exposición a los aerosoles de origen marino. La densidad del bacterioplancton varió de forma considerable entre los diferentes lagos (0,5-6,5x10^6 células mL^{-1}). Estas abundancias obedecieron al estado trófico de cada lago, siendo en cualquier caso relativamente elevadas incluso en los

lagos oligotróficos. Mas allá de la localización geográfica de los lagos, el análisis multivariante (Componentes principales; PCA) realizado con los parámetros limnológicos obtenidos durante el estudio constató la existencia de otros factores, como son la morfología de lago, como explicativos de las diferencias encontradas en el estado trófico de los lagos.

Los lagos estudiados mostraron de forma general una baja diversidad de especies, evidenciando la existencia de redes tróficas relativamente sencillas dominadas por comunidades microbianas. Las únicas especies relevantes de metazooplancton observadas en los lagos fueron el copépodo *Boeckella poppei* y el anostráceo *Branchinecta gaini*, siendo *B.poppei* la única de ambas con un papel relevante en la zona pelágica. La exploración limnológica de los lagos evidenció la existencia de unas comunidades planctónicas microbianas dominadas por pequeños flagelados (tanto heterótrofos como autótrofos), amebas y algunas especies de ciliados. En particular la población de ciliados se compuso mayoritariamente de la especie planctónica *Balanion planctonicum*, de la cual se observaron densidades cercanas a $3x10^3$ ind L^{-1} tras la desaparición de la cubierta de hielo en alguno de los lagos. El fitoplancton estuvo compuesto principalmente de diatomeas pennadas, pequeñas crisófitas, picocianobacterias y clorófitas. En cualquier caso, estas poblaciones no fueron estrictamente pelágicas debido a la re-suspensión del sedimento y/o los aportes de la cuenca.

Uno de los principales objetivos de la tesis ha consistido en comprobar la aplicabilidad de ciertos aspectos de la ecología trófica en hábitats funcionalmente tan sencillos y sometidos a un fuerte control físico. Las redes tróficas pelágicas de estos lagos están truncadas, siendo el copépodo *B.poppei* el depredador superior. A pesar de ello, nuestras primeras exploraciones sugieren la existencia de una intensa relación trófica entre este copépodo y el bucle microbiano. En la tesis se expone un modelo conceptual del funcionamiento ecológico de uno los lagos ubicados en el altiplano de la península (Lago Limnopolar), adoptado para este caso como modelo de estudio. Mediante la realización de bioensayos en los que se manipularon artificialmente las densidades de zooplancton y la concentración de nutrientes, se pudo constatar la existencia de una potencial cascada trófica. La eficiencia de dicha cascada trófica estuvo determinada por el control de las poblaciones de protozoos por parte de *B.poppei* mediante depredación (control *top-down*). El efecto indirecto de esta interacción biótica fue el aumento de las poblaciones de los organismos picoplanctónicos, tanto heterótrofos como autótrofos, debido a la relajación de los bacteriovoría. Los experimentos demostraron como dichas interacciones se canalizaron principalmente a través del control de las poblaciones de ciliados, cuya

abundancia se redujo notablemente en respuesta al aumento de la densidad de copépodos adultos. Por otro lado, se observaron también cambios en la estructura de tamaños del bacterioplancton como consecuencia de la bacteriovoría. Otro mecanismo biótico capaz de explicar en parte la abundancia y/o actividad del bacterioplancton es el relacionado con el virioplancton. En el Lago Limnopolar, este virioplancton fue más abundante en las capas profundas, tanto en términos absolutos (6.93-13.7x10^6 VLP mL^{-1}) como relativos a la densidad de bacterias (3.47-7.29), mostrando además una correlación significativa con la densidad de copépodos.

Los resultados obtenidos en los bioensayos mostraron también la capacidad de este copépodo de promover un reciclaje efectivo de nutrientes, lo que sugiere la incorporación de dicho mecanismo en el modelo de funcionamiento de la cadena trófica del lago. En este sentido, los movimientos de migración vertical llevados a cabo por *B.poppei* y observados durante alguna de las campañas, sugieren también como posibilidad la existencia de una translocación de nutrientes, acoplada a estos desplazamientos, desde las capas profundas del lago a aguas más superficiales. Esto último estaría en conformidad con el supuesto papel predominante del bentos en el funcionamiento del lago. Para explorar estos aspectos, se llevó a cabo un estudio del fraccionamiento isotópico del carbono en distintos compartimentos del Lago Limnopolar. Los resultados indican que *B.poppei* se beneficia principalmente de recursos pelágicos. Por otro lado, el análisis por cromatografía líquida (HPLC) de los carotenos taxón-específicos revela la capacidad de este copépodo para provocar una redistribución de las abundancias relativas de los distintos grupos del fitoplancton. Dicho mecanismo está regulado por una depredación diferencial, la cual depende de la susceptibilidad de la presa a ser ingerida. Así, los resultados de la cromatografía indican un efecto positivo del zooplancton sobre las clorófitas (e.g., *Ankistrodesmus antarcticus*) y negativo sobre las diatomeas o algas crisófitas. Existen sin embrago en nuestro caso otras observaciones que sugieren un comportamiento nectobentónico de esta especie, lo que estaría en consonancia con lo observado en otros lagos antárcticos y que apuntaría a una cierta plasticidad fenotípica de este copépodo. Por el contrario, en el caso del anostráceo *Branchinecta gaini*, el fraccionamiento isotópico parece ajustarse más con un uso preferente de recursos bentónicos, lo que a su vez pondría de manifiesto la existencia de una segregación de nichos entre los dos metazoos dominantes del lago.

Los ecosistemas situados en altas latitudes son considerados sistemas altamente sensibles a los cambios del clima. Los estudios realizados en la tesis ponen de manifiesto la alta variabilidad existente en las características de la cobertura de hielo de los lagos de la región (grosor, opacidad y duración) como

respuesta a pequeños cambios meteorológicos, lo que permite considerar a este como un factor ambiental altamente sensible. Se han observado por ejemplo diferencias en la duración de la cobertura de hielo entre veranos australes consecutivos de 55 o 25 días. Teniendo en consideración el alcance de estas variaciones climáticas, en nuestra opinión estas deberían ser tenidas en cuenta al tratar de dar explicación a dinámicas y patrones observados a largo plazo y atribuidos meramente a un cambio climático global. En relación a estas dinámicas, se llevó a cabo un estudio parcial del balance calórico en el Lago Limnopolar, observándose las diferencias en la regulación del mismo en función de la presencia o no de la cobertura de hielo. En ausencia de dicha cobertura, se percibió cierta demora en la perdida de calor sensible (Q_S) cuando los niveles de radiación solar fueron relativamente bajos y hubo una presencia constante de vientos, hecho en parte atribuible a la batimetría del lago. Por el contrario, durante los periodos en lo que la superficie del lago estuvo congelada, los únicos movimientos de agua se debieron a corrientes baroclínicas debidas a un calentamiento diferencial de distintos estratos de la columna de agua. Los cambios físicos asociados al hielo determinaron en parte la dinámica del plancton. En presencia de hielo, la disponibilidad de luz y nutrientes son factores altamente limitantes. Durante este período, el picoplancton autótrofo llegó a representar hasta un 50% de la biomasa total del fitoplancton en capas profundas del lago, lo que contrasta con el papel más limitado que generalmente tienen en estos lagos. Por el contrario, tanto el bacterioplancton como el fitoplancton de mayor tamaño, mayoritariamente crisofíceas y diatomeas, muestraron un papel más relevante tras la desaparición del hielo, hecho que coincide con los periodos de mayor turbulencia, así como de disponibilidad de luz y nutrientes. En asuencia de hielo, y aún siendo minoritario, el picoplancton autótrofo en los lagos aumentó sus densidades de forma relativa, i.e., en proporción a la concentración de clorofila-a, conforme disminuyó el contenido de nutrientes.

En general, este fitoplancton presentó biomasas consistentemente bajas en todos los lagos, a pesar de lo cual el componente heterótrofo fue relativamente importante. Lo que sugiere el fraccionamiento isotópico en el Lago Limnopolar es la existencia de aportes alóctonos de carbono capaces de mantener en parte la producción secundaria. En las cuencas de algunos de los lagos existen comunidades bentónicas, mayoritariamente tapetes microbianos, que podrían estar realizando esta función. Esto concuerda con la idea extendida de que los sistemas altamente oligotróficos tienden a tener un metabolismo netamente heterótrofo. La concentración de carbono orgánico disuelto (DOC) medida en los lagos durante la primera campaña de estudio fue de alrededor de 1 mg L^{-1}, la cual parece suficientemente alta como para sostener la actividad bacteriana. Asimismo, estos

aportes de carbono pueden provenir también de los musgos bentónicos como se ha sugerido anteriormente. Los resultados también apuntan a que este bacterioplancton podría mantener una relación de competencia con el fitoplancton por la asimilación de nutrientes minerales, lo que en principio es posible en sistemas tan pobres en nutrientes.

En la tesis se aborda también, mediante el uso de varias técnicas, el estudio de la diversidad estructural y funcional de algunas de las comunidades microbianas bentónicas presentes en Byers. En particular, se realizó un estudio comparativo de tres tapetes microbianos representativos de la diversidad observada en la zona. Dos de los tapetes (*soil mat* y *pond mat*) se ubicaron principalmente en la meseta central de la península, mientras que un tercero (*stream mat*) se encontró de forma mayoritaria asociado a los sistemas lóticos de la zona costera, en particular en los márgenes de los cursos de agua. Tanto las observaciones al microscopio como el análisis mediante cromatografía líquida (HPLC) de la composición pigmentaria de los tapetes evidenciaron una mayor dominancia de diatomeas en el caso del *stream mat*. Por el contario, las comunidades fotótrofas de los tapetes del altiplano (*soil mat* y *pond mat*) estuvieron compuestas mayoritariamente por cianobacterias filamentosas (*Leptolyngbya* sp., *Oscillatoria* spp., *Phormidium* spp., *Porphyrosiphon* sp., *Nostoc* sp.). Se determinó también la actividad fotosintética de estos tapetes, la cual fue comparable en magnitud a la observada en otras comunidades antárticas similares. En cualquier caso, esta estuvo condicionada por los factores de estrés a los que se encontraba sometido cada tapete. Los tapetes *soil mat* y *pond mat* del altiplano proliferan respectivamente sobre suelos parcialmente húmedos o en zonas totalmente encharcadas. A diferencia del tapete de la zona costera (*stream mat*), los primeros presentaron scitonemina en su composición pigmentaria (pigmento anti-UV), un mayor contenido de sustancias exopoliméricas (EPS) y cierto desequilibrio en su composición estequiométrica (C:N:P), todo lo cual indica un cierto nivel de estrés ambiental. Las tasas de asimilación de carbono, principalmente vía fotosíntesis oxigénica, variaron en los tres tapetes entre 2,7 y 4,2 $\mu g\ C\ cm^{-2}\ h^{-1}$, siendo las más elevadas las observadas en el *stream mat*. Los micro-perfiles llevados a cabo con sondas de oxígeno demostraron que la actividad fotosintética tuvo lugar principalmente en capas sub-superficiales del tapete. Estos estratos inferiores mostraron además una composición estequiométrica (C:N:P) más equilibrada en comparación a la capa superficial. Se estudiaron también las tasas de asimilación de distintas formas de nitrógeno en estos tapetes. Únicamente se detectó fijación de nitrógeno atmosférico (N_2) en los tapetes del altiplano, siendo esta además notablemente superior en el *soil mat*. Por el contrario, las tasas de

asimilación de nitrógeno combinado, nitrato y en particular amonio, fueron por unidad de área más elevadas en el *stream mat*.

En la tesis se ha abordado también el estudio de distintos biofilms fotosintéticos que se desarrollan en los cursos de agua de la zona costera, cuya ubicación y características estructurales difieren del tapete observado también en esta zona (*stream mat*). Concretamente se estudiaron cinco biofilms con una distribución muy restringida, asociados a un salto de agua situado en la salida de uno de los lagos (Lago Turbio). Su disposición en el cauce no fue arbitraria, siendo esta una respuesta a la presión ejercida por la corriente y el estrés hídrico, lo que indirectamente parece condicionar también la disponibilidad de nutrientes. En consecuencia, el contenido de sustancias exopoliméricas (EPS), composición elemental (C:N:P) y estructura de la comunidad fotosintética, deducida esta última de su composición pigmentaria, varió de forma significativa entre los distintos biofilms, poniendo también de manifiesto la existencia de un estado nutricional desigual entre ellos. La distribución del biofilm dominado por clorófitas (*Ulothrix* sp.) estuvo restringida al canal central del curso de agua, lo que indica una adaptación a flujos de corriente elevados. Las comunidades dominadas por cianobacterias (*Oscillatoria* spp., *Phormidium* cf. *autumnale*, *Leptolyngbya* sp.) mostraron por el contrario una estructura, actividad y distribución diversa, estando sometidas a una variedad de condiciones más amplia. Las diatomeas dominantes en estos biofilms fueron *Fragilaria capucina* s.l., *Nitzschia cf. gracilis, Nitzschia inconspicua, Chamaepinnularia gerlachei, Planothidium delicatulum y Gomphonema sp.,* estando esta última más asociada a los biofilms sometidos a mayor estrés hídrico. Las tasas de fotosíntesis oxigénica medidas en estos biofilms variaron entre 0,7 y 3,4 μg C cm^{-2} h^{-1}. Las más elevadas son comparables en magnitud a las observadas en otras regiones de la Antártida marítima. Por el contario, las tasas más bajas, medidas en aquellos biofilms sometidos a mayor estrés, están en el rango de las determinadas en comunidades de cianobacterias de la región continental de la Antártida.

En la tesis se evalúa también en que medida la disponibilidad de recursos puede regular la estructura y funcionamiento de la comunidad fotosintética de un tapete microbiano. Para ello se llevo acabo un experimento de adición de nutrientes, combinando de forma factorial la disponibilidad de nitrógeno y fósforo inorgánicos. Dicho experimento se llevó a cabo con el tapete descrito en la zona costera (*stream mat*), observándose que un incremento de la disponibilidad de fósforo favorecía el crecimiento de las cianobacterias en relación a las diatomeas, ambos constituyentes mayoritarios de este tapete. La manipulación experimental no generó en cualquier

caso una acreción detectable del tapete, aunque sí se observó un estímulo de la actividad fotosintética cuando la disponibilidad de ambos nutrientes fue equilibrada. Una aproximación experimental de estas características, constituye a nuestro entender el primer intento de estudiar los efectos del enriquecimiento de nutrientes en la estructura y función de un tapete microbiano en la Antártida.

Los resultados obtenidos demuestran la existencia de distintas interacciones bióticas capaces de jugar un papel clave en la estructuración de estas cadenas tróficas, a pesar del fuerte control físico al que estos ecosistemas están sometidos. Esta idea difiere en parte de la opinión extendida que presupone una ausencia de control biótico en ambientes extremos. En el presente estudio se ha descrito como la dinámica de la cobertura de hielo y otros factores asociados a esta explican en gran medida la sucesión de las poblaciones planctónicas, sin embargo, otros factores como son la estructura de tamaños de la comunidad o la presencia de zooplancton, pueden también determinar ciertos aspectos de las mismas, influyendo de este modo en la respuesta que toda la comunidad muestre a las perturbaciones. Con respecto a los tapetes microbianos, los resultados obtenidos en el experimento de enriquecimiento sugieren que un cambio en el ciclo regional de nutrientes podría alterar también el equilibrio metabólico de estas comunidades microbianas. Los sistemas acuáticos de esta región se encuentran perturbados únicamente por el clima y procesos naturales de eutrofización, no estando directamente afectados por factores de estrés antrópicos. La consecuencia de esto es que estos ecosistemas acuáticos pueden resultar muy eficaces como centinelas del cambio climático, ya que son muy sensibles a los cambios e integran en su funcionamiento distintos procesos que ocurren en la cuenca. En consecuencia, los estudios limnológicos deberían continuar en Byers para así profundizar en las consecuencias del cambio climático tanto a nivel local como a escala global. El conocimiento adquirido con este estudio limnológico refuerza además la idea de establecer esta región de la Antártida como un lugar de referencia internacional para la investigación en zonas polares.

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1. General introduction

1.1. Background

Ecological research explores factors affecting the function and structure of biological communities. It investigates the associations between biological populations and the environment for link effects to their specified causes. One approach in Ecology is to use routinely collected data to determine relationships between variables and general organising patterns. However, to better test causal hypotheses, it is also appropriate to carry out intentional manipulations which can provide better insights into the interactions occurring in the ecosystem. This experimental approach allows us to disentangle the individual influence of factors which correlated under natural conditions.

An important part of ecological research deals with the trophic relations among organisms, which merely implies the construction of links among elements at multiple trophic levels in a network-like manner. Different trophic levels constitute a food web, through which the biomass and energy are transferred. Along this food web, both inorganic and organic compounds are transformed, respired and accumulated. Here the body masses of organisms, predatory relations and metabolic processes are closely related features (GAEDKE 1993). Therefore, one of the first steps to understand food webs functioning is to quantify the abundances and biomass distribution of different organisms. Schematically, these abundances are a function of resources availability and predators' activity. In relation to carbon transfer, pelagic food webs are among the most ''efficient'' food webs in the world (ELSER AND HESSEN 2005). These food webs' structural complexity is a trend that is closely linked to community stability and is an important focal point in the theoretical ecology research agenda (BELGRANO 2005).

Limnology, in particular, studies inland water bodies and the related ecosystems. It embraces the research of these aquatic ecosystems' biological, physical-chemical and geological attributes. The aquatic ecosystem's physical and chemical characteristics largely determine the kinds of organisms that can survive in it. Somewhat differently to the sea, a lake's environment offers a higher level of habitat diversity (PADISÁK 2003). Accordingly, it is possible to find lakes vastly varying in terms of their overcoming radiation, nutrient status, autotrophic/heterotrophic metabolism or food web structure. Furthermore, these features are expected to operate on different spatial and temporal scales, and to affect the chemical and biological process occurring in a lake. In terms of other respects, lakes must be studied by appreciating their historical development and interactions with their surrounding catchment (REYNOLDS 2003). In this sense, lakes

are transitory in nature and some of their characteristics change over time. Additionally, some in-lake characteristics such as bathymetry should be taken into account. A lake's thermal regime, for instance, is supposed to be largely controlled by its morphology (LEWIS 1982). Moreover, lake morphology accounts for the relative importance of pelagic and littoral/benthic habitats. Finally, direct or indirect human influence may accelerate the ecological processes occurring in the lake.

Antarctica is the most undisturbed region on our planet. The continent's climate harshness greatly controls biological processes. Some of the major stresses with which biological communities are threatened are temperature, resources availability (e.g., water, light, nutrients), and rapid regional climate changes. Climate, for instance, controls communities functioning as it regulates the hydrological cycle, thus a cyclic variation in the environmental conditions takes place. However, the climate scenario differs between Antarctic regions and seasonal variations can become pronounced in some of them, thus allowing water circulation. In the ice-free zones of Antarctica, there are different types of water bodies (rivers, lakes, ponds, etc.) which remain as liquid during short periods of the year. It is in these aquatic ecosystems where the most exclusive Antarctic non-marine organisms thrive. By extending the study of these communities, we will improve our understanding of the interaction among climate, biology and the evolution of Antarctica in a global scenario. It will allow us to anticipate the impacts of climate change by considering that these ecosystems are further exposed to climate alteration risks (SUN AND HANSEN 2003; MEREDITH AND KING 2005; QUAYLE ET AL. 2007; STEIG ET AL. 2009). Polar ecosystems' relative simplicity is a major advantage in ecological research works. Particularly, the food webs of Antarctic lakes provide simplified systems in which to disentangle the drivers of the ecological process occurring in them, as well as the potential consequences of perturbations. Most of the terrestrial environments in Antarctica, including inland waters, are young ecosystems, which makes them valuable sites to obtain additional information about evolutionary and adaptive processes.

1.2. Freshwater ecosystems in the Antarctic continent: an overview

Freshwater habitats in Antarctica include lakes, ponds, rivers and streams. They provide a rich diversity of habitats and comprise some of the most important environments in this continent. They support the growth of several types of organisms, rendering an important baseline for environmental studies. These

freshwater ecosystems are useful models to understand microbial, biogeochemical and ecological processes. Reduced inertia in the response to climate perturbations is expected in them. For this reason, they can serve as a useful baseline to study the impact of predicted global warming. The ecological study of freshwater ecosystems in Antarctica expands trophic interactions (HANSSON 1992; CAMACHO 2006a), nutrient limitation (PRISCU 1995; BELL AND LAYBOURN-PARRY 1999), and adaptation to low temperatures (PANDEY ET AL. 2004; MORGAN-KISS ET AL. 2006). In particular, the Antarctic lakes that preserve the ice cover the whole year round are potentially considered analogues to lacustrine environments in the early phases of planet Mars (ANDERSEN ET AL. 1994, MCKAY ET AL. 2005), which emphasises their scientific interest. There are several lake districts in Antarctica in which limnological studies have been conducted on a regular basis (Table 1.1). In general, these sites are ice-free areas located in coastal regions (Fig. 1.1). They are sites that have partly undergone deglaciation because of mountains blocking the continental ice sheet. The research conducted at these sites has contributed significantly to the knowledge of the functioning of high latitude freshwater ecosystems. Further reading about some of these sites is offered in Table 1.1.

Most of these ice-free areas are part of the so-called Antarctic "oases". The use of this name began in the first International Geophysical Year, although its international acceptance dates back to the 1980's (SOKRATOVA 2007). These "oases" are sited around coastal locations (Fig. 1.1) and show distinctive geochemical and biological features (MATSUMOTO 1998). As a common trend, the geomorphology of these sites is determined by periglacial landforms. They present numerous temporary shallow melt-water ponds and permanent lakes in which relatively complex communities develop (LYONS 1997). In general, they have a particular drainage pattern, which is determined by the high rates of ablation that take place during summer periods. The lakes located on them can vastly differ in accordance with their hydrological regime and physical characteristics, thus providing a wide range of lake habitats which can vary from entirely freshwater to hypersaline. It is possible to differentiate three biogeographical zones in the Antarctic Continent: the sub-maritime, the maritime and the continental Antarctica. The continental Antarctica includes the eastern side of the Antarctic Peninsula south of 63º S and the rest of the continent. The freshwater environments in this region are distributed circumpolarly, which triggers a colder, drier climate if compared to the maritime region (ELLIS-EVANS 1996a).

1.2.1. Continental region

Continental lakes are morphologically diverse; however, a harsher climate confers them greater stability in the limnological cycle. Persistence of ice produces an effective barrier upon them against external factors. One of the most important sites in the continental region is the **McMurdo Dry Valleys**, which comprise the main ice-free area of the Antarctic continent (~ 4,000 Km^2). It is an arid region located in the Transantarctic Mountains of South Victoria Land. Because of this southern location, the lacustrine ecosystems in this area are among the most extreme Antarctic ecosystems. The site has several perennially ice-covered lakes, many of which are meromictic (so-called because they never completely mix). These lakes are hydrologically connected with glaciers through a network of streams. Given this permanent ice cover, they are not influenced by wind-induced turbulence. The limnological research in the site has been conducted by New Zealand and US scientific national programmes for a long time. Most of this research is part of the interdisciplinary Long-Term Ecological Research (LTER) program funded by the US National Science Foundation (http://www.lternet.edu/), specifically in Taylor Valley. Given its features, these freshwater ecosystems represent environmental limits of the LTER gradient. Microbial mats are also widely distributed in this area, lining ponds and shallow lakes with different chemical and biological characteristics (HOWARD-WILLIAMS ET AL. 1990).

Other important areas in the continental region are the **Vestfold** (68°33′S 78°15′E) and the **Larsemann Hills** (69°20′–69°30′S 75°55′–76°30′E), whose biological diversity is somewhat greater when compared to the Dry Valleys. The Vestfold Hills include a high concentration of meromictic lakes, which are likely to be the largest in the world (GIBSON 1999). The Larsemann Hills are a 50-km^2 free-ice area which also contains hundreds of freshwater lakes of varying sizes, depths and biology (ELLIS-EVANS ET AL. 1998). There, some lakes have evolved to fresh or brackish lakes, and receive periodic influxes of salt water from the sea spray and surges produced by glacial calving (GILLIESON 1991). The deepest parts of numerous lakes from this region are lined with cyanobacteria-dominated microbial mats. These benthic communities show different physiognomies according to the lake morphometry (SABBE ET AL. 2004). Metazoan zooplankton is notably sparse in the lakes from this region. Nonetheless, some species such as cladoceran *Daphniopsis studeri* Rühe has been found to actively predate on the algae and bacteria in Lake Druzhby (SÄWSTRÖM ET AL. 2009).

Table 1.1. *Summary of some of the most important lake districts in Antarctica.*

Area	Location	Representative lakes	Selected bibliography
Mcmurdo Dry Valleys	77°30'S, 162°E	Bonney, Fryxell, Vanda	(FOUNTAIN ET AL. 1999; PRISCU 1998)
Signy Island	60°43'S, 45°38'W	Heywood, Sombre	(QUAYLE ET AL. 2002; BUTLER ET AL. 2005; PEARCE 2005)
King George Island	61◦54'- 62◦16'S 57◦35'- 59◦02'W	Tres Hermanos	(UNREIN AND VINOCUR 1999; VINOCUR AND PIZARRO 2000; VINOCUR AND MAIDANA 2010; CALLEJAS ET AL. 2011)
Vestfold Hills	68°33′S 78°15′E	Ace, Druzhby, Highway, Organic	(DARTNALL 2000, BOWMAN ET AL. 2006; SHEREE ET AL. 2011)
Larsemann Hills	69°20′–69°30′S 75°55′–76°30′E	Crater Lake, Nella, Long Lake	(GILLIESON 1991; GILLIESON ET AL. 1991; ELLIS-EVANS ET AL. 1997, 1998 ; GASPARON AND BURGESS 2000 ; SABBE ET AL. 2003)
Peninsula Byers	62°40'S, 61°00'W	Chester Cone, Midge Lake	(JONES ET AL. 1993; ELLIS-EVANS 1996b)
Amery Oasis	70-72°S, 64-69°E	Beaver, Radok, Terrasovoje	(LAYBOURN-PARRY et al 2001; WAGNER AND CREMER 2006)

Close to the Vestfold and the Larsemann Hills is the **Amery Oasis**. It is sited to the east of Antarctica (70–72ºS, 64–69ºE) and comprises a large deglaciated area (~1,800 km^2; ADAMSON ET AL 1997). The limnological research into the Amery Oasis has been conducted principally by Australian and German expeditions. Lakes at the site usually lose all or most of the ice cover for a short period in late summer. This region is home to Lake Radok, the deepest (~360 m) non-subglacial lake in the Antarctic continent, with an area covering approximately 150 km^2 (WAGNER 2003). It is connected to Beaver Lake, which is located in the centre of the Amery Oasis. It is an ice-covered epishelf lake of 50 km in length and is 25-30 km wide (CREMER ET AL 2004). Otherwise, one particular case is the **subglacial lakes** sited below the icecap. They are more common in the continent than previously suspected, and are nowadays documented to be around 145 (PRISCU ET AL. 2005). Using seismic and radar surveys, it has been possible to map the outline of these lakes and to measure the depth of the water. Main examples are Lake Vostok and the more recently discovered Lake Ellsworth (SIEGERT 2000, SIEGERT ET AL. 2004). The majority of

them are 3-5 km long (SIEGERT ET AL. 2007), although some largely exceed this length, which is the case of Lake Concordia (30 km) or Lake Vostok (250 km).

1.2.2. Maritime region

The maritime Antarctica comprises the Antarctic Peninsula and the surrounding islands of the Scotia Arc. This region is characterised by a less extreme climatic regime, which results in a large number of freshwater ecosystems which are ice-free during the austral summer (ELLIS-EVANS 1996a). Lakes, ponds, sea-pages and streams are typical features of the landscape. These ice-free areas are characteristically small (not larger than 100 km^2) with small-sized watersheds. In these areas, it is particularly evident that the lakes represent the integration of different landscape-related aspects. For instance, they are especially sensitive to watershed variations such as freezing-thawing cycles and snow accumulation over several periods (QUESADA ET AL. 2006).

The **Fildes Peninsula** (62º 12'S, 58º 54'W) is an important limnological area on King George Island. It is a rockery ice-free area located in the south-western part of the island. In general, the nutrient contents in lakes from this site are rather low. The considerable environmental impact caused by tourism in the region has led to propose the site to become an "Antarctic Specially Managed Area" (ASMA) by the German Antarctic Programme. Other lake districts on King George Island are the **Potter Peninsula** (62°14'S, 58°39'W) and the **Barton Peninsula** (62°13'S, 58°47'W). Another important site in the maritime region is **Signy Island** (60°43 S, 45°38 W), located approximately 900 km south-west of South Georgia. Signy Island is part of the South Orkney Islands, which lies within the northern limit of the maritime region. This site has been largely studied by the British Antarctic Survey (HEYWOOD 1967, HEYWOOD ET AL. 1980), evidencing a wide variety of lake types. Unlike depauperate continental lakes, most lakes from Signy Island hold abundant populations of microcrustacean grazers. Two lakes that have been closely studied in the region are Heywood and Sombre. The former is located on the east coast of the island. It has two basins, covering an area of $4.5x10^4$ m^2, and a maximum depth of 6.4 m (BUTLER 1999). This lake is a good example of natural eutrophication promoted by marine fauna (seals), resulting in dense phytoplankton growth in spring and summer. It makes water become turbid, which prevents benthic vegetation growth (ELLIS-EVANS 1990). The freshwater ecosystems from **Byers Peninsula** are slightly similar to those from Signy Island. This ice-free area is located to the west of Livingston Island (South Shetland Islands) and shows an elevated concentration of non-perennial ice-covered lakes and streams. From a limnological point of view,

this region is the most important site of the South Shetland Islands, and probably of the entire Peninsula region. However, knowledge of the limnology of Byers Peninsula is still scarce despite its potential scientific interest (ELLIS-EVANS 1996b).

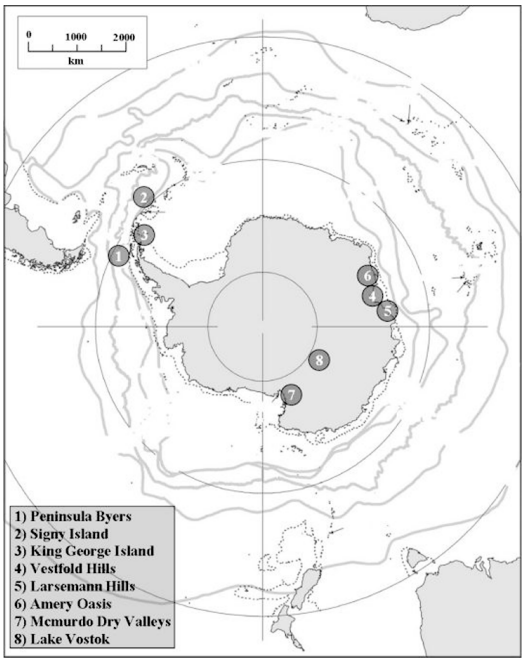

Figure 1.1. *A map of the Antarctic continent showing the location of the lake districts mentioned in the text. Adapted from TRATHAN ET AL. (2007).*

As mentioned before, Antarctic freshwater ecosystems can be considered the ends of an environmental gradient. In this sense, they are largely affected by latitude-dependent factors. It must be noted, however, how the harshness of the climate is not uniform across the entire continent. Thus, the maritime region can be judged as a humid region if compared to continental locations as precipitation in the former reach values close to 600 mm per year (Fig. 1.2), resulting in the presence of abundant liquid water during summer. This climatic gradient makes these regions valuable for studying the effects of hypothetical regional warming, and for allowing critical assessments of biological changes associated with this. Otherwise, Antarctic lakes are far from direct human influence. In other regions on the Earth, climate change and human stresses often interact, producing complex interactions that are not easily approachable. The complexity of these interactions is lower in Antarctica given its remoteness and isolation.

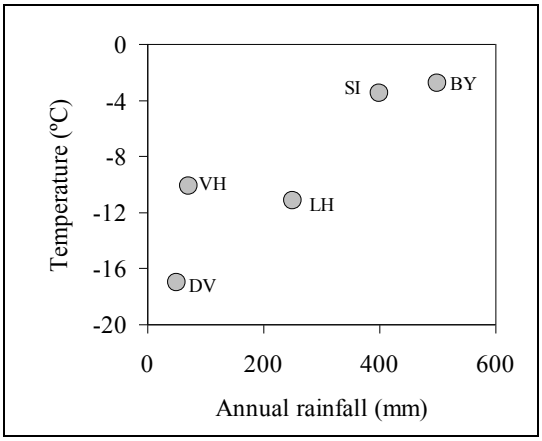

Figure 1.2. *The climatic conditions of different Antarctic regions for which limological research is relevant. The data are mean annual temperatures from different sources: the Vestfold Hills, Bureau of Meteorology Australian Government (*http://www.bom.gov.au*); McMurdo, International Station Meteorological Climate Summary, Version 4.0, Signy Island, VINCENT W.F. (2004); Byers Peninsula, (BAÑON 2001); King George (FERRON ET AL. 2004); the Larsemann Hills (Sabbe et al. 2004).*

1.3. Antarctic freshwater biota: components and communities structure.

In general, Antarctic biota exhibits a high degree of endemism. The taxonomic groups' diversity and abundance differ so much from anywhere else in the world (ROGERS 2007). In lakes in particular, this fact is strongly determined by light availability, temperature and freeze-thaw cycles. Consequently, lakes in the Antarctic continent are to a large extent low productive and microbial-dominated ecosystems in which fishes are absent. The planktonic food webs of these lakes are truncated, showing assemblages nearly composed of viruses, bacteria and protists and a few zooplankters (LAYBOURN-PARRY AND PEARCE 2007). This fact involves the occurrence of simplified trophic structures at which the crustacean metazooplankton, when is present, generally occupies the superior consumers' position (CAMACHO 2006a).

Antarctic lakes are regularly oligotrophic ecosystems (Table 1.2). Far from human influence, the chemical composition of Antarctica lakes basically responds to regional climate and catchment geology. Yet, the eutrophication promoted by marine fauna can be significant in some cases (LAYBOURN-PARRY ET AL. 1996, BUTLER ET AL. 1999, IZAGUIRRE ET AL. 2001). On the other hand, nutrients availability typically increases because of the ice thaw and snowmelt runoff, but also

from internal loads which accumulate in the lakes' bottom sediments. Major nutrients such as nitrogen and phosphorus may also originate from atmospheric deposition (either rainfall or snowfall) which, in relative terms, can be an important source of nutrients as productivity is lower.

In nutrient-poor lakes, the amount of energy and matter circulating through live compartments is reduced when compared to those with a higher nutrient content (HART ET AL 2000). In such circumstances, whatever processes that increase the energy transfer to high trophic levels acquires more relevance and confers great significance to the microbial-regulated pathways, which order a major fraction of nutrient remineralisation (FENCHEL 2008). Bacteria, for instance, use these pathways to connect with the classical pelagic food web by consuming dissolved organic material (DOM). A diversity of protozoans may feed on both bacteria and picoautotrophic organisms, which helps recycle organic matter back into the food chain. Table 1.3 summarises the different sized classes of phytoplankton which may occur in Antarctic lakes.

Table 1.2. *Some limnological parameters obtained in the summer season in some representative Antarctica lakes. Depth, maximum depth in m. Tmax, summer maximum temperatures in ºC. surface PAR, photosynthetic active radiation in µmol m^{-2} s^{-1}. Chl-a, chlorophyll-a concentrations in µg L^{-1}. NPP, net primary production in µg C L^{-1} day^{-1}. Extracted from THOMAS ET AL (2008). Districts are: Vestfold Hills (VH), McRobertson Land (ML) and DV (Dry Valleys).*

Lake	District	Depth	Tmax	PAR	Chl-a	NPP
Crooked	VH	1.0	4	320	0.2-1	0.3-38.6
Ace	VH	4.5	1.2-6	566	1.3-5.0	22.8-195.6
Highway	VH	2	0.8-1.2	216	0.6-2.4	8.2
Beaver	ML	6-8	0.22-0.55	105-205	0.05-0.4	1.5-6.9
Vanda	DV	10	3	80	0.05	0.13
Fryxell	DV	5	2-5	6.13	6	>30
Bonney	DV	4	1		1.21	0-3.2

The idea that trophic relations in aquatic ecosystems work as a web rather than a simple linear food chain derives in part from the acceptance of the microbial loop as an integral part of the complete system (AZAM ET AL 1983). The microbial loop is the way by which the biomass is routed into the classic food chain via bacteria and their grazers. This concept thus implements the idea of a simple food chain. In this sense, the former involves a higher structural intricacy than that expected for a simple linear chain of energy passage. The schematic of figure 1.3

illustrates the microbial loop pathways involved in the pelagic food web of a typical freshwater lake. As commented before, different protozoa, such as amoebae, ciliates and flagellates, graze on pico-sized organisms (SHERR AND SHERR 2002; WEISSE 2002) and are important to conduct carbon from the microbial loop to the higher trophic levels. Recently, viruses have begun to be considered important players of these microbial loop pathways by increasing DOM cycling. In the Mediterranean Sea, for instance, they have been found to sustain bacterial diversity and to control prokaryotic production in combination with the grazing exerted by flagellates (BONILLA-FINDJI ET AL. 2009).

Table 1.3. *Different sized classes of plankton (after Summer 1994) which may occur in Antarctic lakes.*

Size of group	Lower limit (µm)	Upper limit (µm)	Examples in Antarctic assemblages
Femtoplankton		<0.2 µm	Viruses, some bacteria
Picoplankton	0.2	2	Almost all the bacteria, picocyanobacteria
Nanoplankton	2	20	Heterotrophic and autotrophic nanoflagellates, and some ciliates
Microplankton	20	200	Rotifers and some ciliates
Mesoplankton	200	2,000	Copepods

1.3.1. Bacteria

A large portion of the carbon incorporated into the lakes food webs, mainly in the unproductive ones, is through the bacterial utilisation of allochthonous DOC. These bacteria represent a main food source for the microheterotrophic food web (both ciliates and nanoflagellates). Some studies suggest the allopatric evolution of bacterial taxa in Antarctica (TINDALL ET AL 2004). Indeed, several new bacterial species have been reported in lakes from the continent in the last few decades (KHARE ET AL 2009). As with other aspects, the application of culture-independent molecular techniques has allowed us to observe the existence of changes in bacterial populations in accordance with the nutrient conditions in Antarctic lakes (PEARCE 2005, SCHIAFFINO 2009, VILLAESCUSA ET AL. 2010). Besides, bacteria are believed to be an important source of novel biochemicals such as low-temperature enzymes and anti-freeze proteins (LAYBOURN-PARRY AND PEARCE 2007).

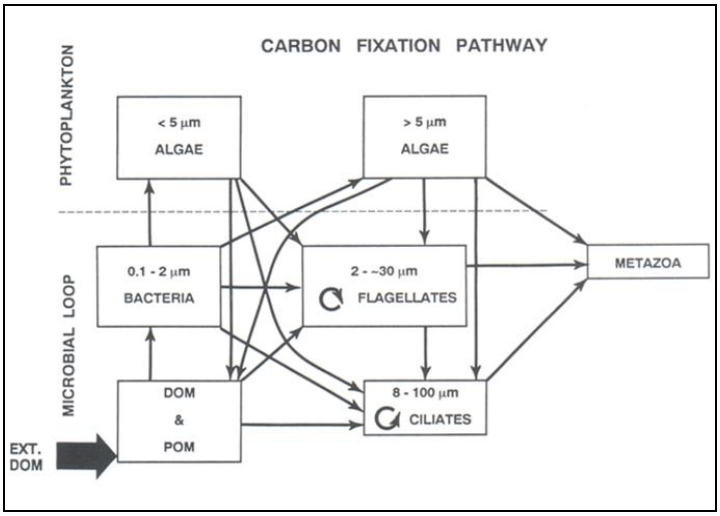

Figure 1.3. *Schematic diagram showing the pools and fluxes of biomass in a pelagic food web. Extracted from* WETZEL *(2001).*

1.3.2. Phytoplankton

Among the smaller (0.2–2 µm) photosynthetic pelagic organisms we find picocyanobacteria. They are ubiquitous and often represent a major fraction of primary production in lakes (STOCKNER 1988). This group appears to be dominated by a single clade composed mainly of strains assigned to the genera *Synechococcus* and *Synechocystis*. Both are morphologically similar and exhibit a broad distribution in Antarctic lakes (VINCENT ET AL. 2000). They have been found to dominate in the saline lakes of the Vestfold Hills (East Antarctica), showing densities in the order of 10^6 cells ml^{-1} (VINCENT ET AL. 2000). However, long-term monitoring in this lake and others indicates important shifts in their relative dominance over different years. Their photosynthetic pigments include the red protein phycoerythrin, which is able to capture low levels of sunlight for photosynthesis. Besides cyanoprokaryta, there are also eukaryotic organisms which fall in this size range; for instance, coccal green algae usually referred to as '*Chlorella*-like' cells (WEISSE 1993, WINDER 2009). Pico-sized phytoplankton are found under wide-ranging conditions; however, its importance lie in it declining from ultra-oligotrophic to eutrophic conditions. The minor domain of cyanoprokaryta in phytoplankton assemblages has been also attributed to their lesser capability to progress in acid waters (STOCKNER AND SHORTREED 1991). This picophytoplankton largely enters higher trophic levels via the microbial loop.

Typical genera of the photosynthetic flagellates occurring in Antarctic lakes are *Chlamydomonas*, *Pyramimonas*, *Chroomonas*, and *Ochromonas*. Some of these protists are mixotrophs, namely they are able to combine the phototrophic and phagotrophic nutrition. It allows them to obtain mineral nutrients from sources in which they are often several orders of magnitude more concentrated than in the dissolved phase (e.g., both heterotrophic and autotrophic picoplankton). Otherwise, their short generation times confer them the ability for fast reactions to short-term variation.

1.3.3. Protozoan

Heterotrophic protozoans are recognised as an important component in pelagic food webs. For a long time, it is known that both heterotrophic flagellates and ciliates are important grazers of bacterial production (SHERR ET AL. 1983; ŠIMEK ET AL. 1995). Often this grazing pressure directly regulates bacterioplankton numbers and productivity. These heterotrophic protists may play a key role when metazoan bacterivores are minimal or virtually absent, which occurs in some Antarctic lakes.

Additionally, flagellates may be eaten by ciliates, as observed in some Antarctic lakes (MATALONI ET AL. 2000). They may even compete with metazoan for algal food. All these issues confer them an important role in nutrient remineralisation and carbon flow. To acquire information on their growth and feeding behaviour, it is essential to understand the energy transfer in Antarctic lakes. For instance, if most carbon is reprocessed via the microbial loop rather than the vertical flux, this is because the activity of these protozoan.

1.3.4. Metazoan

Both phytoplankton and small protists are eaten by zooplankton. Larger metazoans are rare or entirely absent in the majority of Antarctic lakes due to climate harshness. Yet it is still possible to observe differences between maritime and continental regions. For instance, only some copepods nauplii have been occasionally observed in Lake Joyce of the McMurdo Dry Valleys (ROBERTS ET AL. 2004). In contrast, copepods (*Boeckella poppei* and *Parabroteas sarsi*), fairy shrimps (*Branchinecta gaini*), and two species of insect (the dipterans *Parochlus Steinenii* and *Belgica antarcticus*) occur in the maritime region (CONVEY AND BLOCK 1996). By feeding on nano- and microphytoplankton and protozoans, metazoans connect the base of the food web to

higher trophic levels. However, due to the Antarctic food webs' truncated character, they virtually represent their top consumers.

These metazoans excrete dissolved nutrients resulting from their grazing activity and are, therefore, being involved in nutrient cycling. Some lakes from the maritime region show metazoan assemblages characterised by an overwhelming dominance of copepod *B. poppei*. This copepod has been previously classified as *Pseudobockella poppei* before being merged with the genus *Boeckella* (BUTLER ET AL. 2005). This species is present in lakes from Hope Bay (IZAGUIRRE ET AL. 2003) or Byers Peninsula (ELLIS-EVANS 1996b). This copepod is the only crustacean to have been found in the water column of lakes from the Amery Oasis (BAYLY ET AL. 2003). The biogeography of this copepod extends also to the lakes of South America and sub-Antarctic islands (MENU-MARQUE ET AL 2000). There are some palaeoecological records that support a post-glacial colonisation of this species from the maritime Antarctic region. Probably, this colonisation started at southern latitudes after the Last Glacial Maximum, some 120,000 years ago (GIBSON AND BAYLY 2000). Nevertheless, these authors note that this is not completely irrefutable because it contradicts the idea that this copepod originates from South American.

1.3.5. Microbial mats

The photosynthetic community of lakes is not only composed by algae suspended in water, but also by flora attached to the sediment (phytobenthos). Microbial mats are among these benthic communities. They are between the most productive and successful of known microbial communities, and are considered the largest non-marine biomass present in polar regions (QUESADA ET AL. 2008). Microbial mats comprise an assemblage of microorganisms involving a functionally integrated system (PAERL ET AL. 2000), which principally includes cyanobacteria, diatoms, and bacteria. The structural organisation of these benthic communities dates back by approximately 3.5 billion years (DES MARAIS 1990). They dispose following a vertical distribution pattern forming cohesive and laminated structures (Fig. 1.4). The physical proximity of microorganisms, although dominated by a few functional groups, allows the development of a large amount of metabolic conversions, which are based on interactions with dissolved and colloidal compounds.

The commonness and ecological fate of microbial mats in Antarctica have been broadly documented (WILSON 1965, NADEAU AND CASTENHOLZ 2000, VINCENT 2000, SABBE ET AL. 2004; JUNGBLUT ET AL. 2005). Indeed, there have been citations for over a century about the occurrence of these communities during the

earliest Antarctic expeditions (MURRAY 1910, TAYLOR 1916). One general convention is that, regardless of mats' slow growth rates, they become dominant in extreme environments because these habitats are unliveable for their competitors and potential predators. They are widely dispersed in several regions of the continent, and are considered responsible for most primary production (VINCENT 2000). It is during the summer season when mats' phototrophic biomass enhances higher productivity. Some research works carried out in the Dry Valleys have revealed how mats can be preserved in a cryptobiotic state and become photosynthetically active with the onset of favourable environmental conditions (DORAN ET AL. 2003; MCKNIGHT ET AL. 2007). In particular, this benthic production might represent a significant input of allochthonous organic carbon for the unproductive pelagic food webs of nearby lakes. For this very reason, they must be judged quite important to high latitude ecology.

Taxonomical studies of microbial mats in the Antarctic Peninsula (VINOCUR AND PIZARRO 1995) and King George Island (VINOCUR AND PIZARRO 2000) have revealed a richer species composition than in continental Antarctica. In terms of biomass, cyanobacteria are generally the dominant members, and there are recent works suggesting a great Antarctic endemism than assumed hitherto (TATON ET AL. 2006). Thus, although diatoms and chlorophyta can also prove important on microbial mats (they can even dominate under particular conditions), filamentous species of cyanobacteria, such as Oscillatoriales of the genera *Phormidium* spp. and *Leptolyngbya* spp., are usually the major photosynthetic constituents. Yet colonial taxa, such as *Nostoc* sp., which is able to fix elemental nitrogen, can abound in some cases. Other cyanobacterial species found in mats are *Gloeocapsa* spp., *Schizothrix* sp. and *Calothrix* sp.

These laminated communities has been suggested to be possible recorders of environmental conditions in the recent past in the Dry Valleys region (SUTHERLAND AND HAWES 2009). This is because the characteristics of the carbonate deposits layered upon them might be linked with variations of some environmental factors. In these cases, only the upper part is photosynthetically competent, whereas mainly calcite crystals over which the mat accretes compose the inferior layers. A topic that remains poorly investigated, however, is the effect that an increase in nutrients availability might have on these communities. This could well have interesting implications in the resource competition theory context, which predicts that resource-supply ratios may competitively regulate the microbial community structure (SMITH 1993); for instance, modifying the relative dominance of the phototrophic groups. In other respects, microbial mats are of further interest

because their analogy with sedimentary laminated structures denominates stromatolites, the Earth's oldest known ecosystems. Consequently, it is important to study them to understand the early stages of the planet's live evolution. Other important characteristics of these communities are that they show a lower albedo if compared to snow and ice. As a result, they increase meltwater, which indirectly might extend the time of the growth season (MUELLER AND VINCENT 2006).

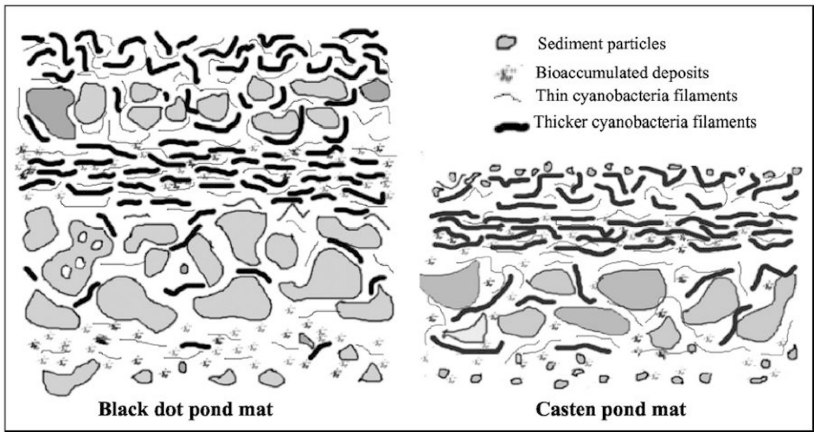

Figure 1.4. *Bi-dimensional vertical scheme showing the typical distribution of microorganisms and mineral particles in a cyanobacteria-dominated microbial mat. Lines of different thickness indicate different cyanobacterial morphotypes. Extracted from CAMACHO AND FERNÁNDEZ-VALIENTE (2005).*

1.3.6. Benthic mosses

Mosses, jointly with lichens, constitute dominant terrestrial vegetation in the ice-free areas of the Antarctic continent. Around 100 species of mosses have been described in Antarctica (OCHYRA ET AL. 2008); however, only a few of them grow submerged. The latter occur in oligotrophic lakes, usually at depths of around 4-5 m, forming well-developed pillars at the bottom (IMURA ET AL. 1999, KUDOH ET AL. 2003, LI ET AL. 2009). This is the case, for instance, of the mid-shallow lakes from Byers Peninsula or some deeper ones in the East Antarctica (WAGNER AND SEPPELT 2006). A relative dominance of phytobentos in relation to phytoplanknton in terms of biomass seems commonplace in these lakes (PRIDDLE 1980, HAWES 1990, IZAGUIRRE ET AL. 1998). Usually, it is possible to find mature populations almost covering the bottom of some lakes. This is because there are no large animals that rely on moss for food. Yet a variety of flora and fauna can be found closely

associated with these aquatic mosses (PRIDDLE AND DARTNALL 1978). These observations imply the necessity to assess the importance of these benthic communities in terms of biomass in Antarctic lakes. On some Shetland Islands, including Byers, it is *Drepanocladus longifolius* (Mitt.) Paris which composes these populations (LI ET AL. 2009), a bryophyte within the semiaquatic Amblystegiaceae (Fig. 1.5). This species occurs also in Signy Island, the South Orkney Islands, James Ross Island and Vega Island on the eastern coast of the Antarctic Peninsula (Fig. 1.6).

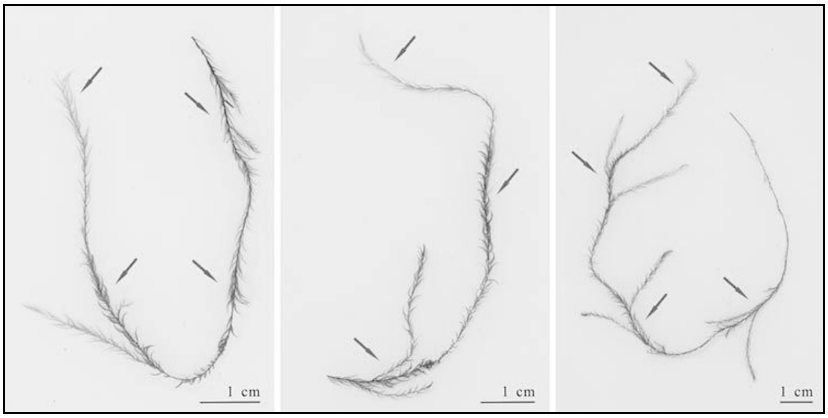

Figure 1.5. *Pictures showing the morphological differences of Drepanocladus longifolius in consecutive winters and summers (arrows indicate growth in different summers). Extracted from LI ET AL. (2009).*

Based on the nomenclature applied to macrophytic life-forms (POKORNÝ AND KVĚT 2004), these mosses are muscids which pertain to the hyphydates group. They are cryptogams which produce spores in their sexual stage. The alternation from reproductive phases can respond to environmental conditions by changing to an asexual stage as stresses increase. In any case, there are several studies indicating that haploid mosses can maintain more genetic variation than that expected (SKOTNICKI ET AL. 2000 and articles cited therein), which supposedly offers great adaptive capability. In contrast to phanerogams, mosses lack internal tissues specialised in water conduction, which implies that nutrients are passively conducted through leaves. They are attached to substrates by rhizoids. The nutrients accumulated during growth are released into the environment after decomposition; however, large amounts are retained in buried parts. In this sense, it has been shown

how phosphorus tends to accumulate in macrophytes in shallow lakes (ROONEY AND KALFF 2003)

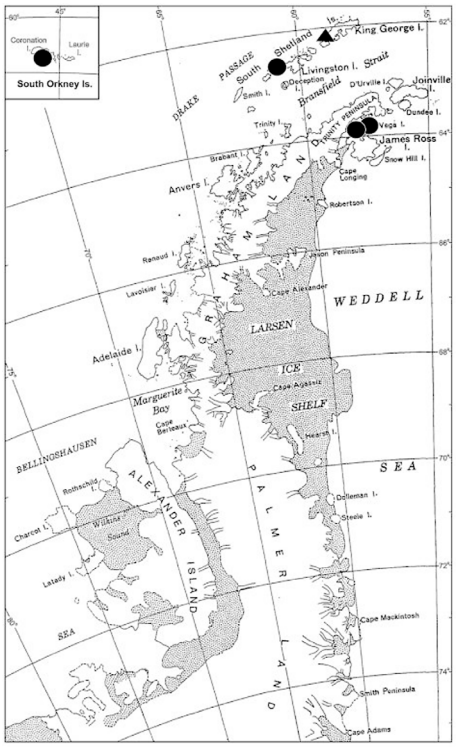

Figure 1.6. *Distribution of Drepanocladus longifolius in the Antarctic. Localions are indicated by filled circles or triangles. Extracted from LI ET AL. (2009).*

Although the benthic organisms in polar lakes have adapted to low irradiance (WALTON AND DOAKE 1987, HAWES AND SCHWARTZ 2000), their primary production can be quite limited at moderately high latitudes (like in Byers Peninsula). This occurs because the number of hours of light is limited during spring, the solar angle is low, and the absorption coefficient is very high over certain periods. Changes in ice thickness, snow cover and ice cap duration are important variations for the light availability of these underwater communities, and potentially modify primary production and, hence, the whole lake metabolism.

1.4. The significance of Antarctic freshwater ecosystems in trophic ecological studies

Antarctica harbours one of the coldest, driest climates on Earth. For this reason, the life conditions are in marked contrast to those observed in temperate regions. From an ecological standpoint, the remoteness of the continent easily isolates the climate signal to perform biological studies. This fact, together with the general simplicity of the ecosystems, provides a unique "laboratory" to study several ecological processes, thus permitting the evaluation of some general trophic and functional ecology questions. Most efforts made to increase the knowledge of Antarctic freshwater ecosystems have dealt with patterns of species distribution and their taxonomic description. However, experimental studies are still scarce. In this sense, manipulative experimentation may prove convenient to understand the role of biotic factors in shaping the community structure.

The theory foresees that the communities controlled primarily by abiotic factors are those far from equilibrium (Table 1.4). In this sense, the prevailing belief is that non-trophic processes regulate ecosystems in Antarctica (MCKENNA ET AL. 2006). Here, the food web complexity (i.e., relative importance of predation and competition) is expected to be lower because of environmental stress (MENGE AND SUTHERLAND 1976). This idea is outlined in the figure 1.7. The food chains in these cases are shorter, thus involving a reduction of energy flow to higher trophic levels. An important feature of these ecosystems is their low buffering capability, implying that impacts, although minor, may be sufficient to significantly affect biological communities. Nevertheless, interactions could be relatively complex, even in those lakes containing simplified food webs.

As mentioned before, all the lakes mentioned in section 1.2, including those from Byers Peninsula, are fishless and can generally be considered unproductive systems with reduced planktonic diversity. The trophic pathways in these lakes largely involve the microbial loop (CAMACHO AND FERNÁNDEZ-VALIENTE 2005). Nevertheless, the presence of some metazoans, such as copepods, rotifers and cladocerans, suggests greater trophic complexity (Fig. 1.8). Indeed, the existence of a potential top-down control in the planktonic microbial food webs from sub-Antarctic and maritime Antarctic lakes with a similar structure to those from Byers Peninsula has been conjectured (CAMACHO 2006a). The occurrence of top-down forces implies the control of population dynamics by consumers which, in addition, may cause trophic cascades. These trophic mechanisms have been extensively studied in temperate regions (CARPENTER ET AL. 2001), but their role in extreme

environments is not yet fully understood. Trophic cascades conduct through the food chain producing a negative and positive outcome alternatively. Therefore, this involves positive feedbacks between non-adjacent trophic levels. In mid-latitude lakes, the cascade extends to three or four trophic links; that is, from piscivorous or planktivorous fishes to phytoplankton through the zooplankton (CARPENTER ET AL. 2001).

Table 1.4. *Ecological gradient form the non-equilibrium ("extreme ecosystmems") to the equilibrium state. Ecosystems and their communities are susceptible to being sited at some point of this gradient. The figure has been taken from (Krebs, 2001, after Wiens, 1984).*

Non-equilibrium	Transitional scenarios	Equilibrium
Biotic decoupling	→	Biotic coupling
Species independence	→	Competition
Unsaturated	→	Saturated
Abiotic limitation	→	Resource limitation
Density independence	→	Density dependence
Opportunism	→	Optimality
Large stochastic effects	→	Few stochastic effects
Loose patterns	→	Tight patterns

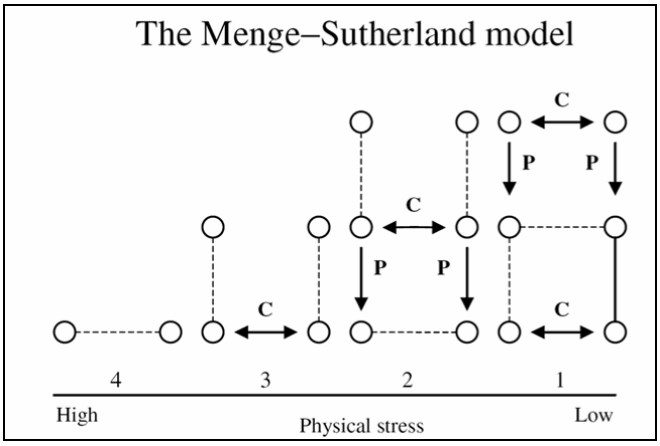

Figure 1.7. *Conceptual scheme showing the Menge-Sutherland model. Letters indicate the interactions between species (C = competence, P = predation). The role of these interactions is supposed to increase as environmental stresses diminish. Extracted from SIGEE (2006).*

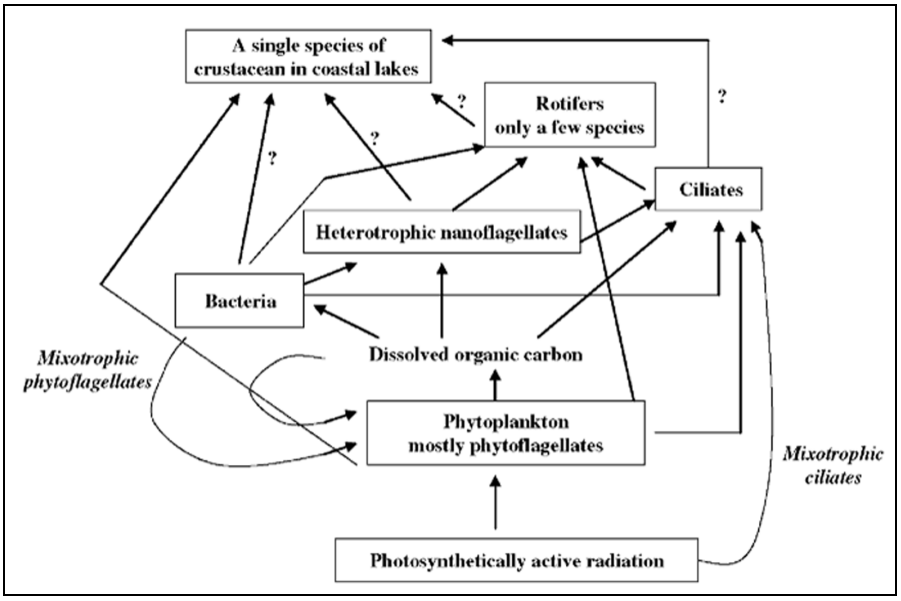

Figure 1.8. *Conceptual scheme showing the planktonic food web of a continental Antarctic lake. The graph illustrates the major role of microbial loop pathways in these kinds of ecosystems, including myxotrophy; however, interactions with metazoans are virtually unknown due to their scarcity. Extracted from Thomas et al (2008).*

A major feature of Antarctic lakes, mainly those from the maritime region, is that the biological dynamics are governed by strong seasonality. The seasonal dynamics of metazoan populations, for instance, depend on temperature and food availability. Therefore, their life cycles must be adjusted to this seasonality in an attempt to be coupled with the onset of primary production. Lakes from the maritime region follow predictable dynamics in relation to light and nutrients regimes. Thus, when the ice covering lakes retreats during summer, conditions are favourable for phytoplankton growth. It is in these periods when zooplankton can obtain many of their requirements to develop. The inter-annual climatic variability in the Antarctic Peninsula region has been found to be higher than in other regions of the continent, which is probably linked to the sea-ice extent (KING 1994). Hypothetically, it may cause an uncoupling between the timing of higher primary production and zooplankton development. One important issue should then be to analyse the feeding patterns of crustacean populations in these lakes by paying attention to their phenotypic plasticity.

1.5. Antarctic freshwater ecosystems in a climatic variation framework

Climate profoundly affects ecosystem functioning. A scientific consensus has been reached on the occurrence of global climate change, although there are some discrepancies about causalities (IPCC 2001, 2007), which has aroused growing interest in the consequences of a global warming. Studies involving the responses of organisms to environmental change should prioritise those regions undergoing faster climate variations. Thus, research into Antarctica is mainly to gain an understanding of the effect that warming has. Several climate models predict that the greatest changes will occur at high latitudes (HOUGHTON ET AL 1995; BARGAGLI 2005). The envisioned scenarios indicate that the climate in some polar regions will be wetter and warmer. Actually, there is a great deal of evidence available indicating that the maritime Antarctic region is suffering warming (SUN AND HANSEN 2003; MEREDITH AND KING 2005; QUAYLE ET AL 2007). More recently, an accurate reconstruction of the temperatures registered in the last 50 years performed by STEIG ET AL (2009) reveals the occurrence of a dramatic warming trend throughout the Antarctic Peninsula (Fig. 1.9). In contrast, it seems that the continental region is cooling as demonstrated by the meteorological data registered in the McMurdo Dry Valleys between 1986 and 2000 (0.7ºC increase per decade), so producing a net fall of temperatures in the whole continent (DORAN ET AL. 2002).

The overall retreat in marginal parts of the ice shelves observed in the region, which is illustrated in figure 1.10, can be attributed to this regional warming (COOK ET AL. 2005). Among the 244 glaciers studied in the study of COOK and co-workers, 87% have shown an overall retreat; the rest have shown an advance; nevertheless, it is small if compared to retreat events. Accordingly, all the glaciers considered in the South Shetland archipelago, where Byers Peninsula is located, showed a retreat without exception. Another interesting outcome of the study of COOK and co-workers was that they verified a progressive southwards migration of this glaciers retreat.

Climate warming involves driving forces acting directly or indirectly on ecosystems functioning. It may cause an increase in glacial run-off and stream discharge, or a thinning of lake ice covers. Regarding lakes, warming affects the timing of run-offs or the supply of essential nutrients from catchments. Furthermore, the non-linear nature of warming outcomes should be noted. Thus, the mean temperature in the next century has been foreseen to increase by around 1.5ºC (IPCC, 2001). Despite this apparently low increase, this would imply major changes

in the freezing and thawing processes in lakes from the maritime Antarctica as typical summer temperatures in this region are slightly above the melting water point. The effect of climate change on some physical aspect of lakes is easily envisioned. An extension of the ice-free period would imply an increase of light availability through the water column and, apparently, a significant nutrient input via runoff, which may induce important changes in lake functioning, e.g. via nutrient fluxes (BLENCKNER 2005), and consequently in primary production (ADRIAN ET AL 1999; PARK ET AL 2004; MCKENNA ET AL 2006).

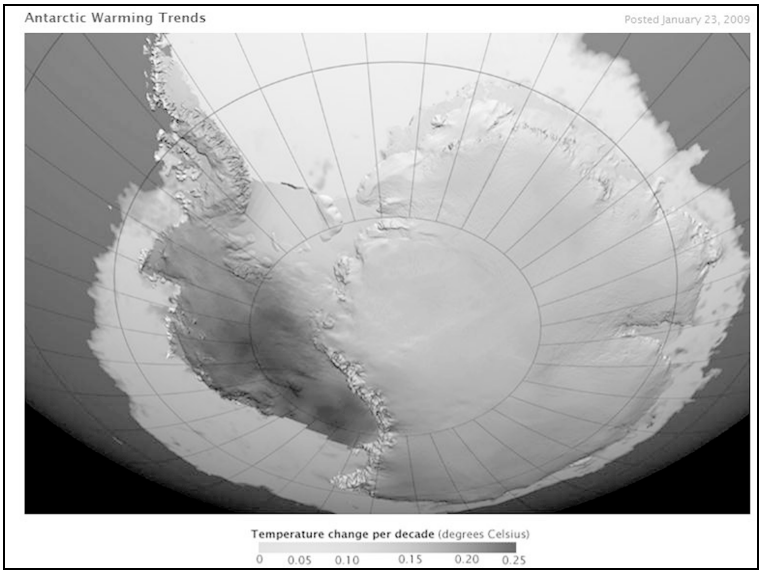

Figure 1.9. *Antarctic warming trends from 1957 to 2006 based on data from weather stations and satellites. Extracted from* STEIG ET AL (2009).

The underwater light regime in polar lakes plays a major role (TANABE ET AL 2008), and it should be considered in connection with the weather conditions of the preceding winter. Thus, attenuation of light through clear ice does not differ substantially from water, but it increases significantly if the ice contains air bubbles or forms irregular crystals upon freezing (WETZEL 2001). Air bubbles' concentration and size depend on the freezing rate in such a way that faster freezing produces greater bubbles density (CARTE 1961). This means that more transparent ice would result from slower freezing processes. Nevertheless, the more important trend regulating the light regime in lakes is perhaps the snow cover of the ice cap, which delays ice-out timing. Whereupon GOLDMAN ET AL (1989) proposed a conceptual

model in which snowfall and total winter precipitation partly regulated primary production during summer.

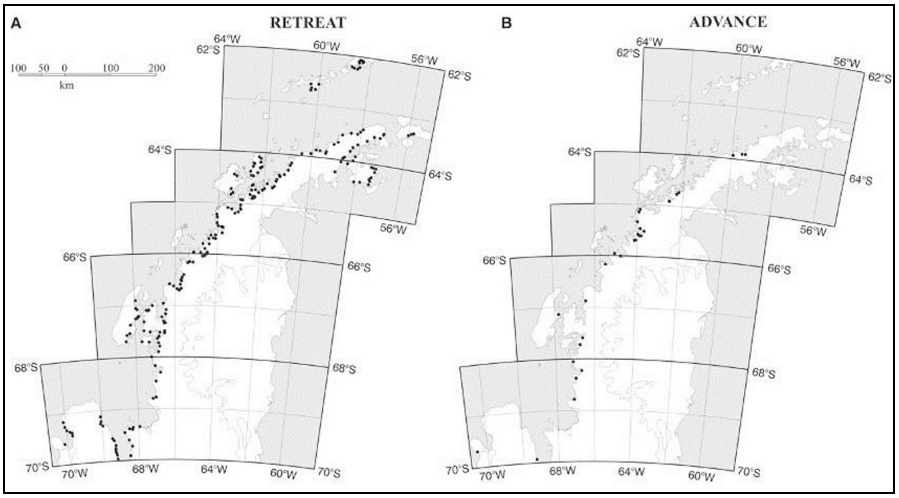

Figure 1.10. *Maps showing the changes (retreat or advance) in the Antarctic Peninsula and associated island glacier fronts for an approximate 50-year period (COOK ET AL 2005).*

The permafrost warming may also affect lake ecosystems. Lake water might drain sub-superficially as a result of permafrost disappearance. A sustained variation in the ground thermal regime in a region might then significantly alter the hydrological cycle. This process has been well-documented in the tundra of northern Siberia using satellite imagery (SMITH ET AL 2005). These authors observed how a significant number of lakes considerably reduced in size, or even disappeared, due to progressive permafrost thawing. It must also be noted how the consequences of warming could be lagged since the permafrost condition in a particular region might not necessarily be in equilibrium with the present climate.

A clear linkage between the advance of deglaciation and the increment of nutrients concentration in some epicontinental waters of the maritime Antarctic region have been recently demonstrated (QUAYLE ET AL 2002). In this study, the authors argue that the thawing that the region has suffered over the last 40-50 years has favoured leaching processes, which has led to increased algal biomass and dissolved nutrients in running waters, in particular phosphorous concentrations (Fig. 1.11). In addition, in a hypothetical scenario with higher precipitation rates, the nitrogen concentrations in water are expected to increase by atmospheric washing.

Actually, an enhancement of precipitation in the Antarctic Peninsula, in parallel with increases in temperatures, appears to have been confirmed by certain observations (TURNER ET AL 1997), thus corroborating some theoretical arguments put forward in previous studies (MANABE AND STOUFFER 1993). It has also been noted how increased atmospheric precipitation involves a higher liquid precipitation if compared to snowfall in both the Antarctic Peninsula region and the South Shetland archipelago (KING 1994, QUINTANA & CARRASCO 2004). This likely implies rain's greater capability to wash contaminants and nutrients from the atmosphere.

In inland waters in which the nutrient contribution via stream inlets and surface run-off is poor, the nutrient supply by atmospheric deposition, either as dissolved or particulate forms, can be of relative importance in controlling nutrient availability (GREENFIELD 1992, VINCENT AND HOWARD-WILLIAMS 1994, BARON ET AL. 2009). There are examples of how these inputs may affect system functioning. In alpine lakes of the Rocky Mountains in Colorado (USA) for instance, nitrogen atmospheric deposition appears to produce a qualitative shift in nutrient supply in lakes to the extent that it changes from a relatively balanced but predominantly N-deficient regime to a more consistent P-limited condition (ELSER ET AL. 2009). Otherwise, SICKMAN ET AL. (2003) observed in Lake Tahoe (California/Nevada, USA) how an increased atmospheric input of nitrogen derives a shift from the co-limitation of phytoplankton by N and P to a persistent P-limitation.

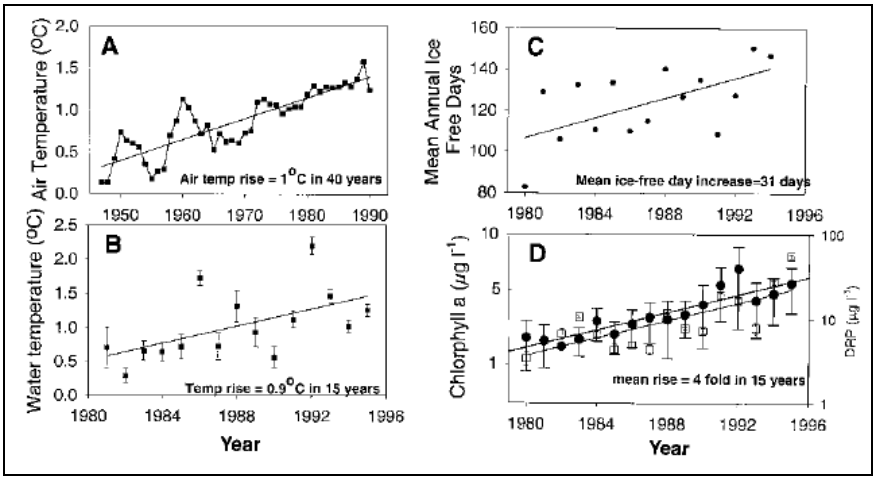

Figure 1.11. *Relation between time and mean winter (July/August) chlorophyll a of the nine Signy lakes on the left y axis (R2=0.5735, P<0.001) and the mean winter (July/August) DRP concentrations on the right y axis (R2=0.8146, P<0.001). Extracted from QUAYLE et al (2007).*

Another subject to be considered is the likely decline in the continent's long-term isolation provided by the Antarctic Circumpolar Current (ACC), which would raise the species transport. As the dispersal of organisms is a function of the connectivity between habitats, the success of colonisation by alien biota might increase if the ACC declines, particularly in the maritime region (FRENOT ET AL. 2005). The susceptibility of the maritime region to receive propagules has been explained to be the result of favourable northerly winds (ELLIS-EVANS AND WALTON 1990). If climate becomes more favourable for non-indigenous species, they will be able to set up stable populations. It is not easy to predict the impacts of these future invasions, although they may hypothetically produce loss of local biodiversity as well as decouples in the trophic interactions. Nonetheless, this matter merits further attention seeing as the isolation of Antarctica has led to somewhat different and particular assemblages of species when compared to anywhere else in the world (ROGERS 2007). On the other hand, whatever the climate changes, the role of humans as an important dispersal factor of foreign biota must be also taken in consideration.

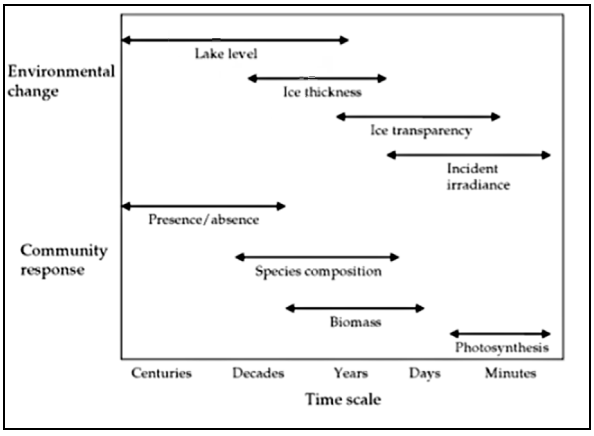

Figure 1.12. *Schematic diagram showing the correspondence between environmental change and community response in benthic microbial communities. Extracted from VINCENT AND LAYBOURN-PARRY (2008).*

Being able to associate biological populations trends with future climate scenarios is a critical issue. Predictions of the physical effects of local climate change in Antarctica appear to have been confirmed, although it is not clear how biological processes will be affected. Hence, our general knowledge is mainly restricted to the short-term context, as point out VINCENT AND LAYBOURN-PARRY

(2008). As these authors indicate, we still need to correctly extrapolate the outcomes of our short-term experiments to a long-term context (Fig. 1.12). It is a difficult task because it is virtually certain that the net result in ecosystem functioning would not be a simple, linear function of warming, but is expected to be the combined effect of multiple factors. It means that some interactions will provide positive feedback which amplifies environmental changes; however, other might potentially offset the warming effects.

1.6. Objectives of this thesis and layout

The research scope of this thesis is extended to the limnological study of freshwater ecosystems in Byers Peninsula. Fieldwork was conducted during the austral summers included in the 2002-2006 period and was undertaken as a part of a broader study programme in the LIMNOPOLAR and LIMNOPOLAR II projects. Both these projects are interdisciplinary research programmes founded by the Spanish Research Programme, which primarily aims to investigate the sensitivity of the polar continental aquatic ecosystems to climate change. Since our major priority is to maintain the primeval environment of the region, all the fieldwork, that is, both sampling and experimentation, has been performed to clearly respect the Antarctic treatise guidelines.

The goals of the thesis are arranged in three specific objectives: 1) Given that Byers Peninsula is an ASPA, we wish to address further information on the biological trends of the area to establish future comparisons with areas that are more violated by human activity. 2) Examine the validity of several hypotheses about the trophic ecology in these ecosystems in order to fully understand how biomass and energy are transferred. 3) Establish Byers Peninsula as an international reference site for monitoring the effects of climate change on non-marine aquatic ecosystems to know the possible usefulness of these environments as sentinels of climate change. In this sense, the outcomes of the present thesis would contribute to gain knowledge on a climatic gradient that spans from the continental Antarctica to more temperate regions on Earth, among which Byers Peninsula is a key area.

The introduction, methodology, results and conclusions sections of the work are set out and developed in 10 sections. The present section (**Chapter 1**) begins by providing a conceptual frame of the thesis. Here some key questions relating to the aquatic microbial communities studied are listed. **Chapter 2** compiles the analytical and instrumental methods used in the thesis, and some particular

procedures are explained in each chapter. This chapter also depicts a general site description and revises some research antecedents of the limnology in the area. The next section (**Chapter 3**) focuses on the site's limnological patterns, provides a general characterisation of several water bodies and establishes the relationships between some of their biotic and abiotic characteristics. **Chapters 4** and **5** specifically consist in the survey of Lake Limnopolar during three consecutive summer periods. To shed more light on the food webs' architecture of these lakes, **Chapter 6** involves field experimental studies to assess the potential role of biotic forces in shaping the planktonic food web structure of Lake Limnopolar. **Chapters 7** to **9** present the study of some of the dominating photosynthetic benthic communities in Byers Peninsula. C**hapters 7** and **8** explore the functional and structural features of microbial mats and stream biofilms, respectively, by assessing their relation with nutrient cycles. The **Chapter 9** includes a field experiment performed with one of these microbial mats for the purpose of checking the effect of nutrient availability on their phototrophic community. **Chapter 10** finishes this thesis by offering a general discussion and concluding remarks. There, some key arguments are also addressed to provide a framework for future researches. **Chapters 3 to 9** are developed similarly, namely they contain an introduction, a description of the particular methodology used in each case, the results and a discussion. Finally, the bibliography referred to in the thesis is compiled.

2. Material and analytical methods

One of the aims of the Regional Sensitivity to Climate Change In Antarctic Terrestrial and Limnetic Ecosystems (RiSCC) programme has been to build a latitudinal gradient in the polar regions. To assist in this objective and wherever possible, we tried to standardize our results with others obtained within this programme by following methods compiled in the manual of Methods for the Study of Non-marine Aquatic Environments (RiSCC 2002). They are standard procedures broadly recognised in regular bibliography. The RiSCC project has been replaced by the SCAR Life Sciences programme Evolution and Biodiversity in the Antarctic (EBA) for 2006-2013.

2.1. Study area

2.1.1. Geographical location and management

Byers Peninsula is one of the largest (~60 km^2) ice-free areas in the Antarctic Peninsula region. As shown in figure 2.1, Byers is sited at the west side of the Livingston Island (62º 40'S, 61º W). This island belongs to the South Setland archipelago, which is located in the southeast side of Drake Passage and holds the greatest number of scientific stations of any region of Antarctica. Livingston Island is jointly with King George one of the great island of the South Setland archipelago. Attending to The Geographic Names Information System (GNIS; http://geonames.usgs.gov/), the Peninsula Byers received its name in 1958 for James Byers, who organized and sent out a fleet of American sealers from New York to the South Shetland Islands in 1820-21. The Byers Peninsula is categorized as one of the sixty-seven Antarctic Specially Protected Areas (ASPA). Byers was designed with the ASPA number 126 at 1966. This recognition was given because of the unusual biological, geological and archaeological values of the site. The Management Plan that designs these ASPA sites has the aim to minimise environmental impacts on these singular regions. The site is pristine and is only open to visits by small numbers of scientists at a time, with stringent environmental protection measures. Be allowed to perform scientific activities within the area requires a permit issued by the Polar Committee.

2.1.2. Geological characteristics

The Byers Peninsula is a periglacial region pertaining to a predominantly volcanic area in which highlight the Precambrian. Byers is the site of the maritime region at which most extensively the rocks from the Mesozoic-Cenozoic magmatic arc complex are exposed (HATHWAY AND LOMAS 1998). Based on the study of NAVAS ET AL. (2005), it is possible to distinguish two main areas in the geomorphology of the site (Fig. 2.2). One of them comprises extensive raised marine platforms and beaches bordering the entire coast. The other area comprises an elevated platform which lay between 85 and 100 m a.s.l. This upland is plentiful of erosive and depositional features of glacial origin. Deposits from Holocene dominate the beaches, while the upland region is composed by non-marine tuffs that pertain to Lower Cretaceous. Leaving out some exceptions, the region shows in general a smooth topography in which only few formations of volcanic origin as the Chester Cone (188 m) and the Cerro Negro (143 m) stand out. Sedimentary material is greatly composed by conglomerates, sandstones and mudstones (Fig. 2.2). Still, it is possible to observe some inclusions in the stratified material (HATHWAY AND LOMAS 1998, DEMANT ET AL. 2004) such as dikes of a basaltic composition (Fig. 2.3).

2.1.3. Climatic regime

The weather in Byers Peninsula is representative of the maritime Antarctic region and contrast greatly with the climate conditions of the continental region. The weather in Byers is characterized by cloudy skies, windy conditions and a relatively high precipitation. The mean temperatures during the summer range 1-3 ºC, with daily maxima up to 10 ºC and daily minima to -10 ºC. During the winter, minimum temperatures can reach -35 ºC and maxima are always below 0 ºC. The rainfall shows annual mean values of 700 to 1000 mm (BAÑÓN 2001; VAN LIPZIG ET AL. 2004), which mainly occur during the summer. Snow accumulates through the winter but a great part melt at summer, although some snow packs persist throughout summer in some accumulation areas (Fig. 2.4). The region shows a characteristically changeable weather, which produces rapid changes in winds and barometric pressure. It is imposed by alternating wind directions, although westerlies or easterlies dominate.

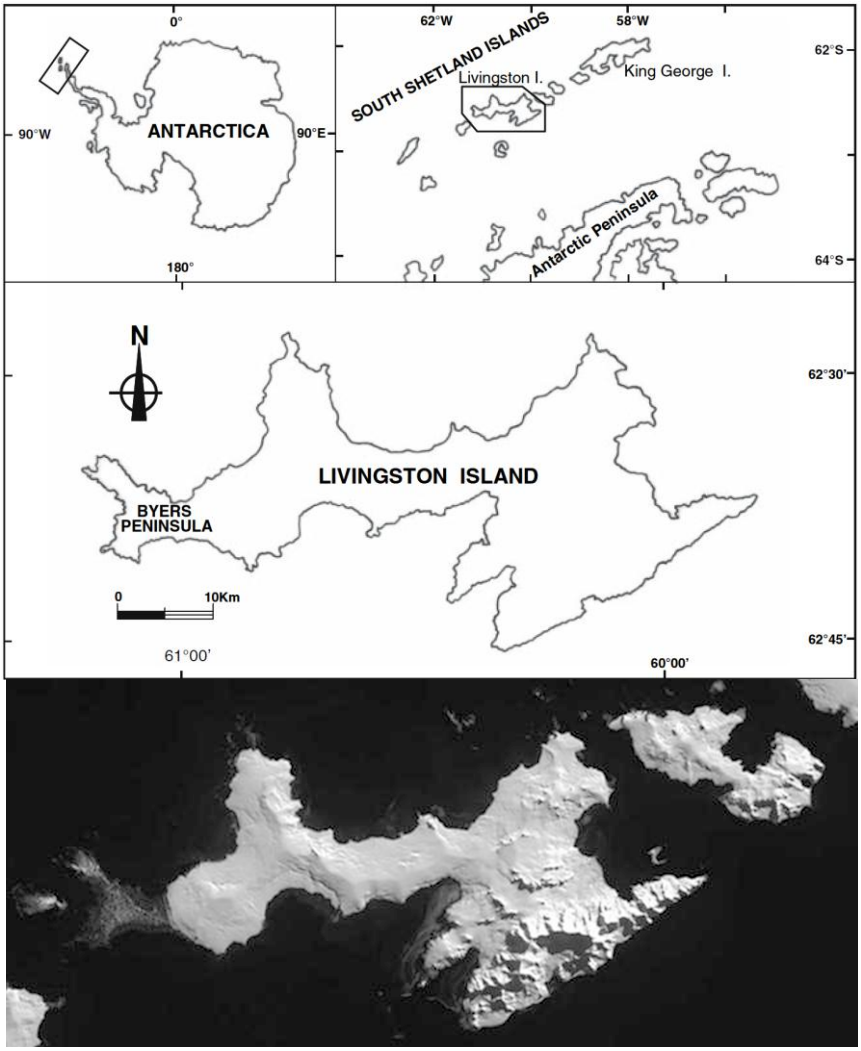

Figure 2.1. *Location of Byers Peninsula (Livingston Island), in the Antarctic Peninsula area.In the satellite picture of Livingston Island it is observed Byers at left as an ice-free area.*

2.1.4. Hydrology

Concerning to its hydrographical features, Byers is characterized by the existence of a complex drainage network, in which numerous lakes, ponds, streams and wetlands cover the land surface (Fig. 2.4). Byers is also particularly rich in wetland areas covered with extensive microbial mats and moss carpets. Attending to the

deglaciation chronology deduced from paleolimnological studies of BJÖRCK and co-workers (1996), a glacier retreat started in Byers 4,500 yr BP, with a considerable part of the region becoming free of ice only 500 years later. The progressiveness of this glacier recoil has originated a gradual distribution of lakes so that more ancient lakes are located toward the east, being also its geographical location and distribution partially organized by the presence of faults and tectonic fractures (LOPEZ-MARTINEZ ET AL. 1996). In nearly all the region, a permafrost active layer is present until around 50-70 cm of depth. These top levels of the soil melt and warm during summer, thus affecting most of the hydrologic and geochemical process occurring in the lacustrine catchments. The predominant sandy soils and gravels further contribute to increase groundwater flows. This dynamic activity virtually lack during winter, when the inlet in the catchment is merely limited to the snow precipitation.

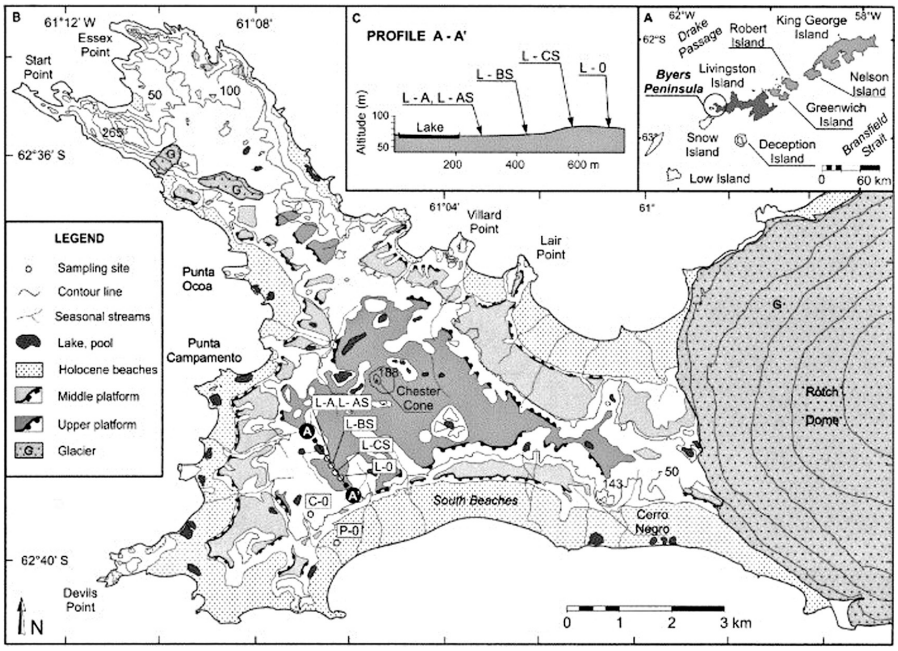

Figure 2.2. *Geomorphological map of Byers Peninsula showing the different sites based on landform and surface deposits. Extracted from NAVAS ET AL. (2005).*

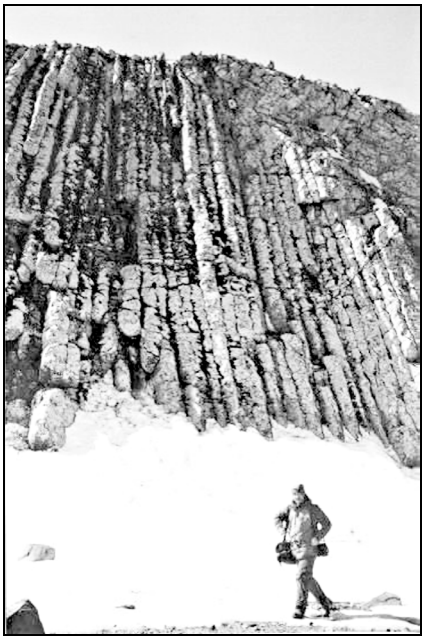

Figure 2.3. *A formation of basaltic dikes located in the vicinities of the Chester Cone (Byers Peninsula).*

2.1.5. Other scientific interests

The vegetation in the catchments of Byers is relatively sparse; still, it is one of the most diverse floristic areas in the maritime Antarctica. The vegetation in the site can be catalogued as characteristic of a tundra type ecoregion. With regards to cryptograms, both mosses and lichens are abundant. The widely extended moss cushions are notorious in the beaches during the summer season (Fig. 2.5). The lichens, represented mainly by the genre *Usnea*, flourish patchily in rocky areas (Fig. 2.6). Otherwise, in Byers occurs the only two phanerogams present in continent (Fig. 2.6), Antarctic hair grass (*Deschampsia antarctica*) and Antarctic pearlwort (*Colobanthus quitensis*). The gradual changes in the distribution of these two vascular plants in the Antarctic Peninsula has been related with warming trends in the region (MATSUMOTO 1998). In other respects, Byers has the most extensive beaches in Antarctica, with rich populations of seaweeds and associated marine life. The site is also a hotspot for marine vertebrates, with several species of marine mammals that reproduce there (well known is the sea-elephant colony) and a large number of birds, including a colony of gentoo penguins (Fig. 2.7). Byers is characterized also by a reach diversity of terrestrial arthropods (RICHARD ET AL. 1994), in which stand out a total of six *Collembola* species (CONVEY ET AL. 1996). Concerning to the archaeology, Byers is unique in possessing the greatest

concentration of historical archaeological sites from Antarctica, which belong to sealers expeditions from early nineteenth century (SMITH AND SIMPSON 1987*;* Figure 2.8). This commercial exploitation of the region began at the early of the nineteen century, just after its discovery at 1819.

Figure 2.4. *Landscape showing components of the drainage network of Byers Peninsula.*

Figure 2.5. *A picture showing the mosses cushions and plants covering beaches of Byers Peninsula during the summer period.*

Figure 2.6. *Terrestial vegetation of Byers Peninsula. a and b) lichens of gener Usnea, c y d) Deschampsia antarctica (Des) and Colobanthus quitensis (Col).*

2.2. Physical and chemical analyses

2.2.1. Meteorological data

Meteorological data in the site were obtained from an automatic meteorological station (AMS; Fig. 2.9) equipped with a Campbell CR10X logging unit, two gel batteries (90 Ah) and one 10 W (0.57 A) solar panel for recharging the batteries. The AMS was located between Lakes Limnopolar and Somero, at 2 km from the coast. The station provided continuous data, with exceptions during some midwinter periods in which battery malfunctions did not provide energy to the datalogger. This station registered the typical meteorological variables as well as water temperature at the bottom of Somero Lake. The data were registered at intervals of 30 minutes. These recorded data consisted of ASCII records containing the mean value of 60 measures (for temperature) or the integration of 1800 measures (for global radiation). Precipitation was measured daily during summer periods (2001 through 2004) by using a simple rain gauge placed about 2 km far from the lakes. In the table

2.1 are summarized the technical specifications of the parameters measured in the AMS.

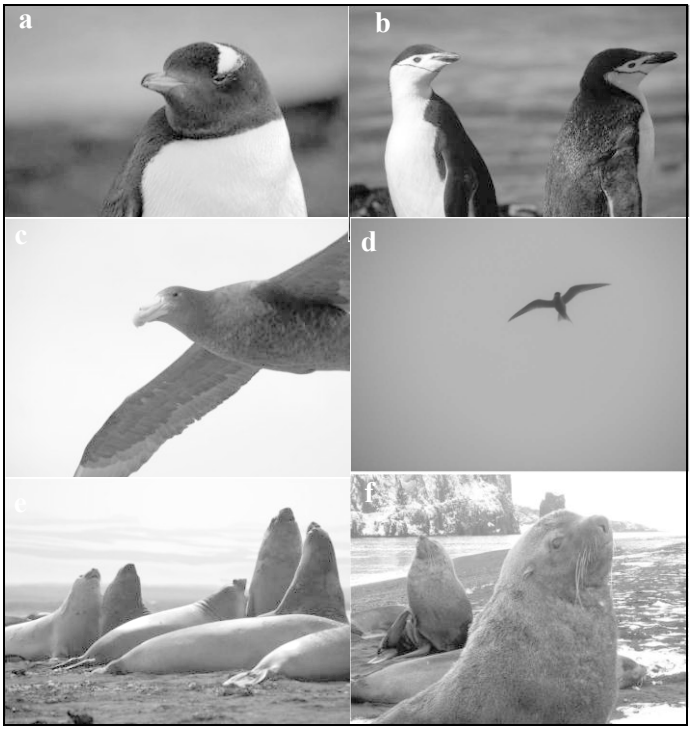

Figure 2.7. *Fauna of Byers Peninsula. a) Gentoo penguin (Pygoscelis papua), b) Chinstrap penguin (Pygoscelis antarcticus), c) Giant petrel (Macronectes giganteus), d) Tern (Sterna sp.), e) Elephant seals (Mirounga leonina), f) Seals (Arctocephalus sp.).*

2.2.2. Oxygen, Temperature, Conductivity, and pH

The water column profiles of oxygen, temperature, conductivity, and pH were performed with a Yellow Springs Instrument (YSI®) Water Logger System multiprobe model 556 MPS with a data output in ASCII engineering units. The multiprobe was equipped with a YSI Precision thermistor for temperature, a steady state polarographic probe for dissolved oxygen (accuracy: 0.2 % and 0.2 mg L^{-1}), a 4-electrode cell with autoranging for conductivity (accuracy: ±0.001 mS cm^{-1}), and a glass combination electrode for pH (accuracy: ±0.02).

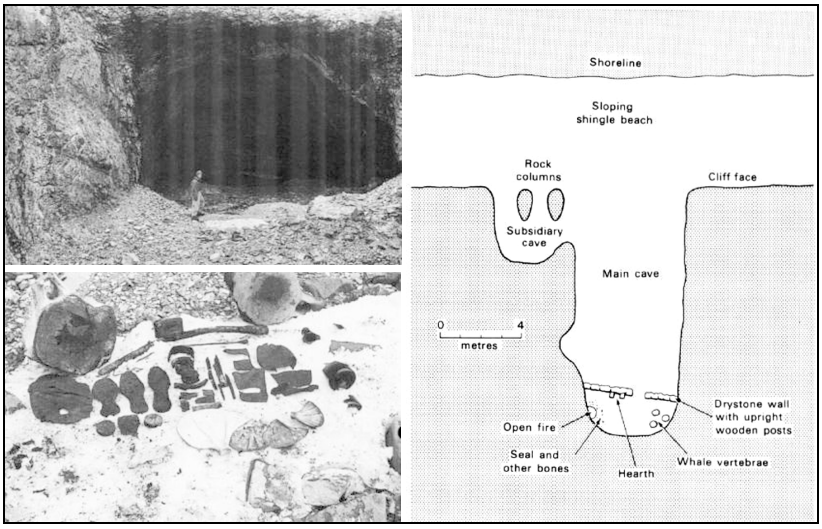

Figure 2.8. *Picture and diagram of a sealers' cave sited in Lair Point (Robbery Beaches, Byers Peninsula). Image on left shows sealers' relics. Pictures extracted from* SMITH AND SIMPSON *(1987).*

2.2.3. Photosynthetic active radiation (PAR)

The photosynthetic photon flux was measured with a 2π scalar irradiance sensor model Li-192SA attached to a LI-COR® datalogger model Li-1000. This sensor has uniform respond in the range of 400-700 nm (PAR). This sensor is a flat receiver that perceives only the zenital light, being so excluded the diffuse radiation from the measures. Differently to the radiation measured in the AMS, in this case the light flux was obtained on the basis of quanta (μmol m^{-2} s^{-1}). When the lake surface was frozen, the light profiles were made by lowering the sensor from beneath the ice. With the data obtained, the extinction coefficients (k_{PAR}) for the ice cap and the water column were calculated in accordance with the equation of Lambert–Beer law:

$$Iz = I_0 \cdot e^{-kz} \quad \text{(Equation 2.1)}$$

$$\ln Iz = \ln I_0 - kz \quad \text{(Equation 2.2)}$$

$$k = \frac{(\ln I_0 - \ln I_z)}{z} \quad \text{(Equation 2.3)}$$

Where *Iz* is the PAR intensity at the depth *z*, and *k* is the extinction coefficient of PAR. Accordingly, it is possible to calculate the extinction coefficient between two depths as follow:

$$k = \frac{(\ln I_1 - \ln I_2)}{z_1 - z_2} \quad \text{(Equation 2.4)}$$

Where z_1 is the depth at the point 1, z_2 is the depth at the point 2, I_1 is the PAR intensity at depth z_1, and I_2 is the PAR intensity at depth z_2.

Table 2.1. *Summary of technical specifications of variables obtained from the meteorological station. Modified from BAÑÓN ET AL. (2006).*

Variable	Unities	Sensor		Measure interval		Observations
		Type	Model	Sampling	Record	
Air temp.	ºC	Pt100	HMP45C	30 s	30 m	Sensor installed at 1.7 m from the ground and sheltered from direct solar radiation
Soil temp.	ºC	Thermistor	107 Probe	30 s	30 m	Sensor installed at 10 cm from the ground and sheltered from direct solar radiation
Water temp.	ºC	Thermistor	Integrated in CS547	30 s	30 m	Sensor installed at 1 m depth
Humidity	%	Humicap	Vaisala Humicap 180	30 s	30 m	Sensor installed at 1.7 m from the ground and sheltered from direct solar radiation
Wind	m s^{-1}		Young 05103	30 s	30 m	Sensor installed at 2.5 m from the ground
Global radiation	Kj m^2	Termo-electric	CM5	1 s	Integrate each 30 m	Sensor installed at 1.9 m from the ground

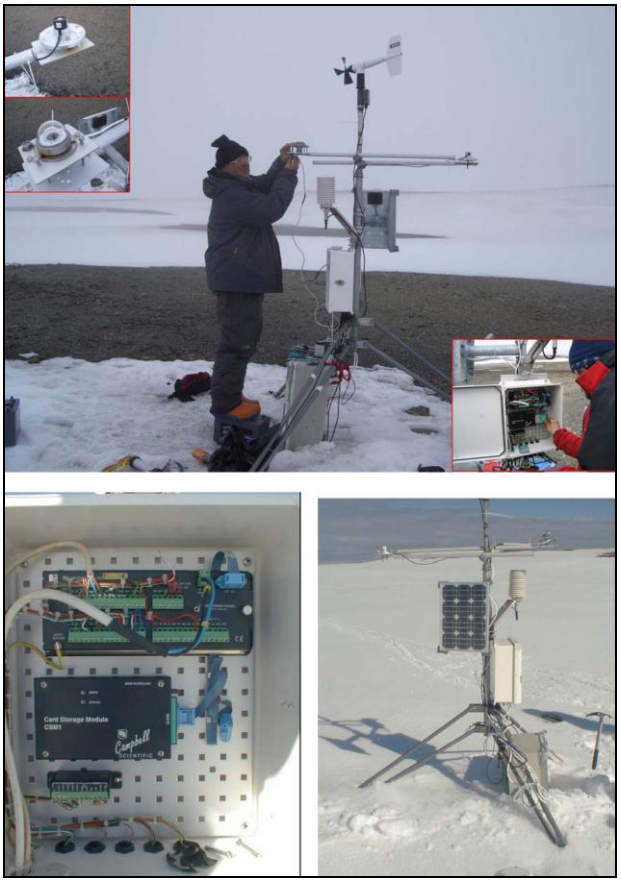

Figure 2.9. *Images of the automatic meteorological station (AMS) installed in Byers Peninsula. Meteorological data are of main importance for the investigation of the terrestrial and limnetic ecosystems within the context of the RiSCC programme. For the determination of the (micro)meteorological characteristics of the geographical area where the research is to be done is recommendable the utilization of these automatic whether stations, which can be programmed to acquire data at the appropriate frequency.*

2.2.4. Mineralization

Alkalinity can be defined as the capacity of water to neutralize H^+ ions and serves as a pH buffer and reservoir for inorganic carbon (MANAHAN 2000). In our case it was measured as total titratable bases and determined by end-point titration following the Standard Methods (2320 in APHA 1992) with HCl using a pH shift indicator (phenolphtalein) of the equivalence end-point pH (pH=8.2). The concentration of major ionic species (Ca^{2+}, Mg^{2+}, Na^+, K^-, SO_4^{2-} and Cl^-) was determined in a Waters capillary ion analyzer (CIA) in the Universidad Autónoma de Madrid.

2.2.5. Nitrogen in water samples

Nitrate plus nitrite (NO_x) were quantified colorimetrically from samples filtered through Whatman GF/F filters following the cadmium reduction method (sensitivity: 40 µg N-NO_3 L^{-1}) described in the Standard Methods (4500-NO_3^- E in APHA 1992; RISCC, 2002). Following this procedure, all the nitrate present in the sample is reduced to nitrite using cadmium granules teatred with copper sulphate and packed in a glass column. Once this, nitrite is determined spectrophotometrically after reacts with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride. The standard solution was prepared with $NaNO_3$.

Ammonium (NH_4) concentrations were obtained from the same samples following the Indophenol blue method (sensitivity: 10 µg N-NH_4 L^{-1}) modified from MACKERETH ET AL. (1978) and listed in the RiSCC manual (RISCC 2002). The method is based in the formation of indophenol blue by the reaction of ammonia with phenol and hypochlorite when nitroprusside is used as catalyst. The standard ammonium solution was prepared with NH_4Cl.

Total dissolved nitrogen (TDN) and total nitrogen (TN; dissolved plus particulate) was quantified from filtered and non-filtered samples respectively by oxidation with alkaline persulphate digestion (NaOH 6 g L^{-1} and $K_2S_2O_8$ 6 g L^{-1} final concentration) at 150 ºC during 2 hours (RISCC 2002). After neutralization of samples, the nitrate present in sample was quantified following the same method described before for nitrate. To get indirect calculations, the sum of NO_x and NH_4 was considered as total dissolved inorganic nitrogen (DIN); and dissolved organic nitrogen (DON) was obtained by subtracting DIN from the total dissolved nitrogen (TDN).

2.2.6. Phosphorus in water samples

Orthophosphate (SRP) concentrations were quantified colorimetrically by the ascorbic acid method following the Standard Methods (4500-P E in APHA 1998; RISCC 2002) from samples filtered through Whatman® GF/F filters. The technique is based in the reaction of orthophosphate with ammonium molybdate and potassium antimonyltartrate in acid conditions to produces phosphomolybdic acid. This compound takes an intense blue coloration when is reduced with ascorbic acid. Minimum detectable concentrations of 10 µg P L^{-1} were achieved using a 5 cm path length cuvette. The standard phosphorus solution was prepared with KH_2PO_4. Total phosphorus (TP) content was quantified in the same way in non-filtered samples

after an acid digestion with sulphuric acid and potassium persulphate (0.072 N and 12 g L^{-1} final concentration respectively) at 150 ºC during 2 hours (RISCC 2002).

2.2.7. Reactive soluble silica in water samples

Silicate concentrations were quantified colorimetrically also from Whatman® GF/F filtrates following the molybdosilicate method (4500-Si D in APHA 1998; RiSCC 2002). The analysis is based in the formation of a complex between silica and ammonium molydate (molybdosilicate). In this method, a solution 10% (w/v) of oxalic acid is added to the reaction to avoid the interference of phosphate in the measure since both molybdophosphoric and molybdosilicate complex are formed indistinctly. The minimum detectable concentrations established for this method are 20 μg SiO_2 L^{-1}. The standard silicate solution was prepared with Na_2SiF_6.

2.2.8. Elemental composition of microbial mats

The quantification of carbon and nitrogen contents in the bulk biomass of microbial mats was performed by the combustion of samples in a CE Instruments EA 1110 CHNS elemental analyser. To prepare samples, they were firstly dried at 60 ºC until obtain a stable weight and then they were ground to powder in a mortar. Thereafter, samples were weighted over aluminium foil sleeves and burnt in the elemental analyser. The gases resulted from burning were measured in an infrared spectrometer as described by KIRSTEN (1983). In parallel with samples, a standard was prepared using sulphanilamide ($C_6H_8N_2O_2S$). Total phosphorus was obtained from dry powder samples following the method described in section 2.1.7.

2.2.9. Quantification of ^{13}C y ^{15}N isotopic ratios

The isotopic ratios of ^{13}C to ^{12}C and ^{15}N to ^{14}N were quantified by isotope ratio mass spectrometry (IRMS) following to FRY ET AL. (1992) with a Micromass-Isochrom mass spectrometer. Isotope values are expressed in delta (δ) notation as parts per thousand deviations from either the international standard V-PDB (Pee Dee belemnite) for carbon or atmospheric nitrogen in the case of nitrogen. Subsequently, the percentage of isotope in sample is obtained using equations 2.5 and 2.6.

$$\%^{13}C = 1{,}11122 + 0{,}0010985 \cdot \delta^{13}C \quad \text{(Equation 2.5)}$$

$$\%^{15}N = 0{,}3664 + 0{,}000363009 \cdot \delta^{15}N \quad \text{(Equation 2.6)}$$

2.3. Biochemical analyses

2.3.1. DOC quantification and CDOM characterization

Dissolved organic carbon (DOC) concentrations were determined by high temperature combustion using a Shimadzu TOC-5000 analyser from natural samples filtered by cellulose nitrate filters with a 0.2 pore size. Potassium hydrogen phthalate ($C_8H_5KO_4$) was used as standard. Additionally, fluorescence measurements were done to characterize and trace the fraction of coloured dissolved organic matter (CDOM) by means of fluorescence excitation emission matrix (EEM) spectroscopy. This technique allows to identify different components of CDOM pool from complex mixtures depending of their optical properties (COBLE 1996; MCKNIGHT ET AL. 2001; STEDMON AND MARKAGER 2005; MURPHY ET AL. 2008).

The method for CDOM analysis is based in the collection of repeated emission scans resulted from excitation at numerous wavelengths. Subsequently, these individual spectra are concatenated to obtain three-dimensional matrix (florescence – emission wavelength – excitation wavelength). The analyses were performed from GF/F pre-filtered water samples, a portion which would be in the range considered such as dissolved matter (0.22-1.22 μm) for DOM analysis (AITKENHEAD-PETERSON ET AL. 2003). Measurements were run on a Perkin-Elmer® Fluorescence Spectrophotometer. Samples were excited from 240 to 465 with increments of 5 nm. For each excitation wavelength, an emission spectrum was recorded over the wavelength range 240–600 nm with data collected every 5 nm. A slits of 10 and 5 nm were used for excitation and emission respectively. The Raman line produced by covalent bound of water was corrected by gross spectral subtraction of Milli-Q water blanks. Based on previously published data, in the table 2.2 are summarized some of these components including a brief description and the positions of their fluorescence maxima. They are designated by letters because their chemical composition is partially uncertain.

Table 2.2. *Positions of the fluorescence maxima and brief description of the different components of CDOM. Secondary maxima are shown in brackets. Ranges of excitation and emission wavelengths are defined based on different published data (COBLE 1996; STEDMON ET AL. 2003; STEDMON AND MARKAGER 2005; MURPHY ET AL. 2008).*

Label	Excitation (nm)	Emission (nm)	Description
A	<250 (305)	398-412	Humic fluorophore group
C	320-360	420– 460	Humic fluorophore group
M	295	398	Marine humic-like
T	275-280	340-344	Tryptophan-like, protein-like
B	275	304-310	Tyrosine-like, protein-like

2.3.2. Exopolymeric substances (EPS) content on microbial mats

The amount of extracellular polymeric substances (EPS) in microbial mats was estimated as the total of their main components (carbohydrates and proteins). For analysis, a known weight from lyophilized samples was placed in a 1.5 ml eppendorf tubes and 1 ml of 2% EDTA was added, subsequently tubes were stirred and heated at 35 ºC during 1 hour. After they were centrifuged at 10000 rpm for 10 min and the supernatant was recovered for analysis. The carbohydrate content of extract was obtained by the measure of hexose equivalents (polysaccharide) content in the sample by the phenol-sulphuric acid spectrophotometric method (HERBERT ET AL. 1971) using glucose as standard. The protein amount was estimated according to BRADFORD (1976) taking bovine serum albumine (BSA) as standard. All assays were carried out on triplicate. For the microscopic observation of exo-carbohydrates distribution in biofilms, formalin 4% fixed samples were dehydrated over glass slides and stained with a Calcofluor White ($C_{40}H_{44}N_{12}O_{10}S_2$; λ Excitation max = 347 nm and λ Emission max = 436 nm) solution during 30 min. Following, the preparations were observed in a Zeiss-III epifluorescence microscopy using the filters setting for calcofluor fluorescence emission. Subsequently, several pictures were taken with an Olympus® C-4040 ZOOM camera and the RGB original images were converted into a grey scale.

2.3.3. Photosynthetic pigments characterization by HPLC and chemotaxonomic determination of algal classes

To analyze the pigment composition of seston, samples were collected in GF/F (Whatman) glass fibre filters and kept frozen until analysis. For microbial mats, cores with a known surface were obtained and keep frozen in whirl-pack bags until analysis. Once in the lab, pigments filters were cut into small strips and extracted with 90% acetone by vortexing and sonication. In the case of benthic samples this procedure was repeated several times until the absorbance of the last extract did not exceed the 1 % of previous extracts. To obtain further concentration of the extracts when necessary their were dried by vacuum before analysis.

Subsequently, samples were pre-filtered by 0.2 μm nylon filters and injected into a Waters® C_{18} Spherisorb S5 ODS2 column (samples of campaign 01/02) or in the same column connected in tandem with a NovaPack C18 column for the rest of the samples. The later column configuration was devised to enhance the photopigment separations. The samples from campaign 01/02 where ran following the procedure described by VINCENT ET AL. (1993), whereas for the rest of samples it was followed a methodology modified from the (PINCKNEY ET AL. 1996) protocol. In our case, Ammonium acetate concentration must to be reduced to 0.1 M to avoid the excess of the pressure in the system. The detailed elution gradient for both chromatographic protocols is shown in tables 2.3 and 2.4 respectively. All the samples injected were composed of a volume of 50-100 μl of the extract mixed with a volume of ammonium acetate (Ion Pairing Agent) to give an IPA final concentration of 0.1 mM. Eluted pigments were detected by a diode array detector (PDA) at a range of absorbance of 350-750 nm.

The peak identities were determined by comparing retention times and spectra with pure standards purchased from DHI®, or with chromatograms from cultures provided by the Department of Biology from the University Autónoma de Madrid. The UV-screen carotenoid scytonemin was identified by comparing the obtained spectra with published data (GARCIA-PICHEL ET AL. 1991). The amount of pigment was quantified against the curves obtained with standards by integration of the area under the cross-section at 440 nm (all carotenoids and clorophylls) or 409 nm (Phaeophytin-*a*). In all cases linear regressions were done with intercept forced to zero. The quantification of scytonemin was made using the published specific extinction coefficient of 45 L $g^{-1}cm^{-1}$ at 440 nm (GARCIA-PICHEL ET AL. 1992).

Table 2.3. *Chromatographic conditions assayed for samples from campaign 2001/02. In the table is shown the elution gradient of solvents in the mobile phase.*

Time	Flow	CH_3OH:CH_3COOHN_4 0.175M	CH_3COCH_3:H_2O
0	1.5	100	0
10	1.5	0	100
30	1.5	0	100

Table 2.4. *Chromatographic conditions assayed for samples from campaigns 2003/04, 2004/05, and 2006/07. In the table is shown the elution gradient of solvents in the mobile phase.*

Time	Flow	CH_3OH	CH_3COOHN_4 0.1M	CH_3COCH_3
0	0.8	80	20	0
5	0.8	80	10	10
45	1.25	80	5	15
50	1.5	80	0	20
65	0.8	80	0	20
67	0.8	80	20	0
95	0.8	80	20	0

Table 2.5. *Mode of detection and taxonomic specificity of some of the pigments after separation through HPLC analysis.* [a]*Cultures were provided by the Department of Biology from the Universidad Autónoma de Madrid*

Pigment	Taxa	Detection
Chlorophyll-*a*	All groups	Standard (DHI®)
Chlorophyll-*b*	Chlorophytes	Culture[a]
Chlorophyll-*c*	Diatoms and Chrisophytes	Culture
Echinone	Cyanobacteria	Culture
Fucoxantin	Diatoms and Chrisophytes	Standard (DHI®)
Lutein	Chlorophytes	Standard (DHI®)
Myxoxantophyll	Cyanobacteria	Standard (DHI®)
Phaeophytin-*a*	All groups	Standard (DHI®)
Zeaxanthin	Cyanobacteria and Chlorophytes	Standard (DHI®)
β-caroten	All groups	Standard (DHI®)

Some carotenoid pigments are specific of different algal classes; therefore, they can be used as unambiguous marker of the occurrence of these algal groups in the sample. Accordingly, the relative contribution to community of different groups,

both in benthic and pelagic samples, was monitored by obtaining their relative amounts (w/w with respect to Chl-a). Table 2.5 summarizes the taxonomic affiliation of different taxa-specific carotenoids used in the study. Additionally, is it shown if they were detected both using standards or by reference cultures.

2.4. Enumeration, sizing, and biomass calculation of planktonic organisms

2.4.1. Virus Like-Particles

Counts of viral-like particles (VLP) were performed following NOBLE AND FUHRMAN (1998). A volume of 0.1-0.5 µl of samples preserved with buffered formaldehyde (2% final concentration) was filtered (<10 kPa vacuum) onto 0.02 µm pore-size Al_2O_3 Anodisc filters (Whatman). Filters were dried completely and stained with a solution of SYBR Green I (Molecular Probes®) using a $2.5 \cdot 10^{-3}$-folds diluted solution of the stock reagent (10,000X concentrate). To minimize the fade produced by UV exposition of sample, slides were mounted with an antifade solution composed by 50% glycerol and 50% PBS (120 mM NaCl, 10 mM NaH_2PO_4 pH 7.5), and 0.1% p-phenylenediamine. In any case, to make shorter the exposition to light, counts were done from digital pictures captured with exposure times around 1 second with an Olympus® C-4040 ZOOM camera. As noted by WEINBAUER (2004), viruses and DNA bound colloids are not readily distinguishable by this technic. Another limitation is that the discrimination between phages and bacteria are based exclusively on size and staining intensity.

2.4.2. Heterotrophic and autotrophic picoplankton

To prepare samples for heterotrophic picoplancton (HPP) analysis, a volume of up to 8 ml of sample preserved in buffered formaldehyde (2% final concentration) was stained with 4', 6-diamidino-2 phenylindole (DAPI; final concentration 0.25 % w/v) and filtered onto a 0.2 µm Isopore GTBP (Millipore). Cellulose acetate backing filters 0.8 µm pore were used to obtain a uniform distribution of cells. After 10 min of exposure to DAPI, samples were filtered with low vacuum (<15 inches of mercury) and mounted on a microscope slide embedded in a drop of low auto-fluorescence immersion oil. To perform counts, a minimum of 20 fields were randomly selected, or 1000 cells were counted from each preparation at 1250x

magnification. For the autotrophic picoplankton (APP) counts, a volume of up to 20 ml was filtered in the same way; but filters were mounted without stain and a minimum of 500 cells were counted at 1250x magnification. The phototrophic cells were visualized by means of green light excitation (BP λ 546 nm , FT λ 580 nm, LP λ 590 nm) according to MACISAAC AND STOCKNER (1993).

For biovolume estimations, numerous microscopic fields were photographed with an Olympus® C-4040 ZOOM camera and subsequently were processed making an edge detection, automatic grey-level thresholding and a conversion to duotone as described in MASSANA ET AL. (1997). After this, a calibration of images was done and the area (*A*) and perimeter (*P*) of a minimum of 500 cells were measured. Finally, the cell volume was obtained by the application of the following equation (BJÖRNSEN 1986):

$$V = \frac{8{,}5A^{2,5}}{P^2} \qquad \text{(Equation 2.7)}$$

When necessary the cells biomass was calculated assuming a constant ratio model (NORLAND 1993) for the conversion of cell volume to carbon of 350 fg C μm^{-3} (BRATBAK 1993).

2.4.3. Heterotrophic and plastidic nanoflagellates

The nanoflagellates counts were performed from samples preserved in buffered formaldehyde (2% final concentration). Nanoflagellates were examined by epifluorescence microscopy. Two different filter sets, for blue light excitation (BP λ 450–490 nm, FT λ 510 nm, LP λ 520 nm) and for green light excitation (BP λ 546 nm , FT λ 580 nm, LP λ 590 nm), were used to discriminate heterotrophic (HNF) and plastidic (PNF, which includes autotrophic and mixotrophic species) forms respectively.

Preparations were obtained by filtering a volume of up to 30 ml of sample with low vacuum (<15 inches of mercury) onto a 0.8 µm Isopore GTBP (Millipore) filters and mounted in slides with low auto-fluorescence inmersion oil. Following, an anataxonomic categorization was made in relation to pigment content (HNF: non-pigmented; PNF: pigmented) and the cell size (small: 2-6 µm long; large: 6-20 µm long). Biovolume calculations were also made; hence, short and long axes of cells were measured with eyepiece reticules of at least 50 individuals and an equation of a

rotational ellipsoid (Equation 2.8, ROBERTS ET AL. 2004) was applied to obtain a mean value for each group:

$$V = \frac{\pi L W^2}{6} \qquad \text{(Equation 2.8)}$$

where V= celular volume (μm^3), L= length (μm) y W= width (μm). A factor of 1.56 was applied to biovolume obtained to correct the shrinkage provoked by formaldehyde fixation (CHOI AND STOECKER 1989). This corrected volume was converted to biomass applying a conversion factor of 220 fg C μm^{-3} for HNF (BØRSHEIM AND BRATBAK 1987) and 200 fg C μm^{-3} for PNF (WETZEL AND LIKENS 1991).

2.4.4. Microplankton

Phytoplankton and ciliate counts were performed from samples preserved in acid Lugol iodine (1.5% final concentration). In the lab, a volume of 100 ml of sample was concentrated by gravity settling in chambers following the Utermöhl method (UTERMÖHL 1958), and inspected in an inverted phase-contrast microscope Nikon® model Eclipse TE2000-S. At least 400 cells were counted per sample.

The detailed analyses for taxonomic identification and counts were undertaken under higher magnification using both differential interference contrast (DIC) and phase contrast microscopy. Dominant phytoplankton forms were identified at least to genus level. Principal taxonomic keys used in identification were ANAGNOSTIDIS AND KOMAREK (1988) and KOMAREK AND ANAGNOSTIDIS (1989) for cyanophytes, FÖRSTER (1982) for conjugatophytes, GERMAIN (1981) for diatoms, and BOURRELLY 1972-1968-1970 for the rest of groups. The mean biovolume for each identified form was obtained using geometrical formulae. Thus, ciliates cell shapes were assumed as nearly spherical ellipsoids using equation 2.8. The different phytoplankton forms were adjusted to geometric forms following the criteria of HILLEBRAND ET AL. (1999). A factor of 1.4 to correct the shrinkage effect due to Lugol preservation was applied (MÜLLER AND GELLER 1993). Volume was converted to biomass applying a conversion factor of 220 fg C μm^{-3} for ciliates (BØRSHEIM AND BRATBAK 1987) and 200 fg C μm^{-3} for phototrophic organism (WETZEL AND LIKENS 1991).

2.4.5. Metazooplankton

The microscopic analysis of metazooplankton was conducted with formaldehyde fixed samples (4% final concentration). The specimens were counted and sized under a Nikon® binocular microscope model TMS. For copepods sizing, the prosome length was measured using an eyepiece micrometer and results were converted to biomass using allometric length-weight (MALLEY ET AL. 1989) and weight-carbon (WEIBE 1988) regressions as follow:

$$DW = 2{,}9946L^{2{,}1951} \quad \text{(Equation 2.9)}$$

$$C = 0.499(DW)^{0.99} \quad \text{(Equation 2.10)}$$

Where DW and L are the dry weight and prosome length respectively, and C is the carbon content in micrograms.

The copepods were grouped into different development stages categories as follow: nauplii, copepodites class I-III, copepoids class IV-V, and adults (class VI). Assignation of individuals to each group was made on basis to the body size. Then, the size ranges delimiting the groups I-III and IV-V were defined using previous data obtained in the site for this specie (DIAZ-MAZIP pers. comm.). Although the size of stage VI was not significantly different to stages IV-V, it was defined by the presence of mature sexual organs. Likewise, naupliar stages were distinguished from copepoids on the basis of morphological trends.

3. Hydrobiological trends in lakes of Byers Peninsula

3.1. Introduction

Antarctic lakes are largely microbial dominated ecosystems (PRISCU ET AL. 1999). The constituents of these microbial assemblages are viruses, bacteria, protists, and a few zooplankton species. Despite of the climatologic hardness, the growth of these organisms may significantly vary under different trophic conditions (PACE AND COLE 1994). Several ecological features depend on the trophic status of water bodies. One of them is the interplay of autotrophic and heterotrophic processes. When inorganic nutrients are not limiting production, the release of dissolved organic carbon (DOC) by phytoplankton may account for the bacterial demand. It implies a co-variation of phytoplanktonic and bacterial production (COLE ET AL. 1988). However, an extreme oligotrophy may result in heterotrophic biomass exceeding that of autotrophs. Different studies indicate that Antarctic lakes are commonly net heterotrophic systems as carbon consumption exceeds its production (Priscu et al. 1999, Säwström 2008). Under these circumstances, the carbon requirements of bacteria can be met by allochthonous inputs from the lake's catchment. On the other hand, the heterotrophic metabolism can be strongly controlled by the supply of inorganic nutrients (FISHER ET AL. 2000). This involves a more complex regulation of lake's metabolism (TEUBNER ET AL. 2003), since bacterioplankton may compete with algae for inorganic resources.

The planktonic food webs of Antarctic lakes have a significant microbial component (LAYBOURN-PARRY ET AL. 1997). At low productive conditions, the running of the microbial loop provides mechanisms to retain nutrients (AZAM ET AL. 1991, WEHR ET AL. 1994), both by reducing the losses produced by sinking and/or by increasing nutrient regeneration through a predation mechanism. Bacteriovorus protists can consume efficiently a large fraction of bacterial production. They, in turn, profit as food for metazoan grazers, which, differently to the continental region, can be also important components of the food web in lakes from the maritime Antarctica (CAMACHO 2006a). By the functioning of this recycling food web, nutrients are made available for the primary producers. However, in an opposite scenario, an increase of nutrient inputs allows a higher contribution of microplankters (AGAWIN ET AL. 2000, BELL AND KALFF 2001), thus enhancing the nutrients burial via sedimentation as a consequence of a production of large and frequently ungrazed phytoplankton.

The nutrient supply determines the biomass production, however, the structure of the food webs are also the result of predator-prey interactions. Thus, in lakes from the maritime Antarctic region (i.e. Signy Island), some variations on the

microbial dynamics and biological interactions have been perceived depending of the degree of animal-induced eutrophication (LAYBOURN-PARRY ET AL. 1996). In that case, a higher nutrient load promoted a higher top-down control on bacterial populations at least during short periods. This might have implications in a scenario of global warming. Hence, far from the human influence, the natural eutrophication of freshwater ecosystems is a gradual process that takes a long time to progress; though, even without a direct human intervention, a regional climate change could greatly accelerate its course (QUAYLE ET AL. 2007).

The limnological studies concerning these issues have hitherto focused in the Dry Valleys area (HOWARD-WILLIAMS ET AL. 1990, HAWES ET AL. 1993, PRISCU 1998), the Vestfols Hills region (ROBERTS AND MCMINN 1996), the Peninsula region (VINOCUR AND PIZARRO 1995, IZAGUIRRE ET AL. 1998), and in the maritime region, particularly in Signy Island (HEYWOOD 1967, 1968, CAULKET AND ELLIS-EVANS 1997; BUTLER ET AL. 2000) or King George Island (DRAGO 1980; UNREIN AND VINOCUR 1999). The designation of Byers Peninsula as an Antarctic Specially Protected Area (ASPA, nº 126) is in part due to its biological significance (see section 2.1). There are indeed previous studies on the limnology of the site, however, these studies focus on benthic habitats (DAVEY 1993, JONES ET AL. 1993) or are broad descriptions which not delve into structural and functional aspects (ELLIS-EVANS 1996b). It is for this reason that a deeper knowledge about the limnology of this region is required.

It is though that the glacier retreat in Byers Peninsula started about 5,000 yr BP and the eastern part of the region became free of ice only 500 years ago (BJÖRCK ET AL. 1996). This deglaciation resulted in a chronosequence of water bodies being at different stages of evolution. Therefore, lakes sited westernmost are supposed to be older compared to those located near the glacier front. Actually, ages of only 400-500 years were established for these lakes (BJÖRCK ET AL. 1996). Lakes from Peninsula Byers might then offer a continuum of limnological conditions. Hypothetically, biological productivity might increase as the lake aging increase because of natural eutrophication. Besides, there are also local factors that can affect lake's productivity, which are related with the hydrologic setting rather than with catchment lithology (i.e., ENGSTROM ET AL. 2000).

In the Antarctic ice-free areas, such as Byers Peninsula, the functioning of aquatic ecosystems is deeply linked with the surrounding land. The landscape-lake interactions are much restricted during winter, however, when the snow melting occurs, interactions become more intense, just coinciding whith the enhancement of the biological activity. Some aspects such as the drainage velocity or nutrients run-

off largely depend of the catchment properties. They are, for instance, size, topography, permafrost dynamics or vegetation coverage. An important trend is also the edaphology of the catchment. At warmer temperatures and higher water availability, the soil development can be favoured. Recent studies undertaken in King George Island outlined a notable accumulation of organic matter in the active layer of cryosols (SIMAS ET AL. 2007), which also might occur in Byers. The terrestrial florain Byers is significant in some catchments, which might facilitae, as observed in other sites, that humus horizons develop with relative facility (VINCENT ET AL. 1988). Although a deep accumulation of peat does not occur in Byers, a 10-20 cm layer of organic matter has been observed in some locations (BONNER AND SMITH 1985). The vegetation coverage in Byers varies among sites from poorly vegetated stony ground (central plateau) to totally carpeted soils (some coastal areas), consequently, differences on soil characteristics among sites can be expected. In a work exploring the phenology of surfaces formation in Byers (NAVAS ET AL. 2006), a conclusion extracted was that the fluctuations of the active layer produced water movements that likely regulates the leaching of soil particles. In other respects, the phosphorus availability in Antarctic catchments can be also related with the soil development, in such manner that younger soils might display a higher availability as observed in Taylor Valley (GUDDING 2003)

Differences in the water chemistry of lakes also depend on the sea proximity, which is known to affect the chemical composition of waters in other sites of continent (Victoria Land, BORGHINI ET AL. 2008). This relies in the salt and nutrients enrichment caused by the sea spray, being the inflows higher nearby the coast. This fact offers a particularity to Byers compared with near locations such as Signy Island, where most of the lakes are located in coastal areas. By contrast, most of the lakes in Byers are inland, thereby increasing its isolation. The proximity to the coast also affects the permafrost active layer deepness (SIMAS ET AL. 2007), which is expected to alter the intensity of the weathering processes. In the aforementioned work of NAVAS AND CO-WORKERS, authors studied different lithological and altitudinal contexts, and found important differences between the platform and beaches of Byers. Concretely, they found gradual increases of pH and carbonates with the coast proximity, thus exemplifing a transitional change between both areas. On the other hand, the ornithogenic soils, both relicts and currents, which in Byers are restricted to the coastal areas (ELLIS-EVANS 1996b), have a higher nutrient content and microbial biomass compared to mineral soils (AISLABIE ET AL. 2009). The importance of these ornithogenic soils is further supported by paleoecological studies such those performed in Ardley Island and Barton Peninsula (LIU ET AL. 2006), in which authors demonstrate how a part of this soils is incorporated into the

lake sediments. For instance, these soils are known to be responsible for the increase of nutrient loads entering in Otero Lake, in the Antarctic Peninsula, after the snow melting (MATALONI ET AL. 2000). It is expected then that trends in the catchment translate finally in particular chemical but also biological characteristics of lakes, which should vary depending of the location.

Here our aim has been to provide a description of nutrient and biological trends in some representative lakes of Byers Peninsula. In spite of the general oligotrophic conditions, it result appealing since this region exhibits water bodies distributed from inland to coastal areas that might contrast in their trophic status. Our study involved the exploration of factors controlling these trophic variations as well the structure of the associated microbial communities, mainly based in the abundances of functional groups and/or size categories, namely pico and nano-sized organisms. We hypothesize here on the relative importance of resource availability in structuring pelagic community regardless of the role of climatic stressors. Admittedly, organisms abundances exclusively does not give an overall description of the energetic mechanism taking place, still, we try to interpret our results also in basis of energetic balances and nutrient fluxes. To attain these objectives, a systematic limnological survey was carried out in some water bodies from the site during the summer 2001-02. Relevant limnological parameters were obtained *in situ* to define the physical structure of lakes. We undertook in parallel a sampling for the chemical and biological characterization. The data relations were explored by using multivariate and regression analyses to identify the main sources of variability between lakes.

3.2. Methodology

3.2.1. Sampling

The survey was conducted during the austral summer of 2001-02 between middle December to early February. Fifteen lakes and a pond were chosen to represent the diversity of water bodies in the site. It was based on their geographical situation, morphometry, and perceptible trophic status. All water bodies were sampled at least once during this period. All measures and sampling were performed on the deepest point of the lake. The vertical profiles of conductivity, oxygen and pH were performed with a CTD probe YSI® every 20 cm. The vertical profiles of broadband

photosynthetically active radiation (PAR; 400–700 nm) were measured with a 2π scalar irradiance sensor (LI-192SA) at 0.5 m depth intervals.

Water samples were obtained with a 5 L Kermmerer hydrographic bottle. Samples for the analyses of inorganic soluble nutrients (NO_x, NH_4, SRP and SRSi) were filtered immediately through Whatman® filters GF/F grade and stored frozen at –20°C in acid-washed PVC bottles until analysis. For total phosphorus (TP) and nitrogen (TN) the same kind of bottles were filled directly without filtration and stored also at –20°C. Additionally, samples were obtained for the determination of dissolved organic carbon (DOC) concentrations in some of the lakes. In this case, samples were taken filtering water by 0.2 pore size cellulose nitrate filters and the water was stored in acid-washed glass vials at 4ºC until analysis.

For the photosynthetic pigments study (chlorophyll-*a* and taxon-specific carotenoids), a volume of water was filtered through GF/F filters, preserved frozen at 20°C and stored in dark until analysis. For picoplankters and nanoplankters counts (both heterotrophic and autotrophic), samples were fixed with buffered formalin (2% final concentration) and kept refrigerated (4 °C) in the dark until the slide preparation. Another sample was preserved in glass bottles with 1.5 % acid Lugol iodine solution for ciliates and microphytoplankton examination. For metazoan sampling, a net mesh sized 50 µm was hauled vertically from the maximal depth to surface. Samples in this case were fixed with formalin (4% final concentration) and stored refrigerated until analysis.

3.2.2. Statistical analysis

Linear regressions were performded with log-transformed data to identify relationships among variables. Correlations were considered to be significant when the probabilities of a Spearman rank correlation were <0.05. In order to explore the variability among lakes, a Principal Components Analysis (PCA) was performed with the abiotic and biotic variables obtained. Data used for PCA's represented the mean values of surface samples from each lake along the entire sampling period. These variables were log-transformed (log10 x+1) to linearize the relationships and avoid the influence of magnitude. The data shown in text and tables are in any case untransformed. In a preliminary exploration of results, some variables were removed from conclusive analysis because they earned redundant information (i.e. oxygen concentration, dissolved fractions of nutrients). According to Kaiser–Guttman criterion (JACKSON 1993), PCA axes were selected if eigenvalues were higher than 1. All statistical analyses were ran using SPSS for Windows Version 15.0.

3.3. Results

3.3.1. Meteorological conditions

In table 3.1 are shown the mean, maximum and minimum values of major climatological parameters recorded during summer 2001/02 in Byers Peninsula. During the period from December 2001 to February 2002, air temperatures ranged from a minimum of -1.9°C to a maximum of 5.0°C, with mean daily values of 1.4°C. Soil temperatures varied somewhat broadly from -3.4 to 8.2. Weather conditions were usually cloudy; in consequence, dial total and PAR radiation averaged 11,596 Kj m^2 and 23.1 µmol m^2 respectively during entire period, showing higher values of 29,952 and 51.1 respectively just before to solstice. The weather was generally wet and windy (westerly winds dominated). Total rainfall from 8th December 2001 to 11th February 2002 was 190.4 mm, with daily measures ranging from 0.1 to 15 mm. The relative humidity was notably and invariably higher, with mean values of 91% and minimum and maximum values of 77 and 98 % respectively. The mean wind during the entire period was 26 Km h^{-1}, although wind velocities around 100 km h^{-1} were occasionally measured.

Table 3.1. *Mean and range values of some climatological parameters recorded in Byers Peninsula during summer 2001/02.*

	mean	max	min
Air Temp. (ºC)	1.4	5	-1.9
Soil Temp. (ºC)	1.4	8.2	-3.4
Water* Temp. (ºC)	4.1	11.7	-1.8
Radiation (Kj m^{-2})	11596	29952	1142
Wind speed (km h^{-1})	26	46	9
Max Wind speed (km h^{-1})	56	104	21
Precipitation (mm)	190.4†	15.2	0

**Probe installed in Lake Somero*

†Total precipitation collected during period (66 days)

3.3.2. Location, morphometry and catchment size of lakes

A total of 15 water bodies (some are shown in figure 3.1), were surveyed in the present study during summer 2001/02. Additionally, a pond sited near sea and closely affected by fauna eutrophication (i.e., elephants seals) was also sampled. The location of lakes and several of their morphometric characteristics are shown in figure 3.2 and table 3.2 respectively. Lakes distributed in two main areas. One of

them comprised lakes sited c.a. 90 m a.s.l. in different points of the central plateau of the Peninsula. These lakes were occupying a landform modified by fluvial and periglacial processes in terrains propitious for water retention. Some of them, such as Chester Cone Lake and Lake Cerro Negro, were located in small catchments near or bounded by isolated volcanic plugs. In general, lakes from this plateau showed clear surface outlets, except in the case of Lake Escondido, which is inclosed by three basaltic hills at the southeast of Chester Cone.

The other set was composed by shallow lagoons located in coastal areas. The water bodies here are in general settled on low relief lands. Concretely, those examined in the present study were located in the South and President Beaches. Their surrounding areas varied in this case from sandy and dry terrains to those largely covered by mosses cushions and plants (see section 2.1.5). Despite of coast proximity, they were totally or partially isolated from the sea by a physical barrier. However, a direct hydraulic connection with sea might occur occasionally such as in Lake Maderos, thus implying a hydrological balance subject to the marine tidal influence. This lake received in addition the outlet of Lake Limicolas, located a few meters to the north shoreline.

The lakes studied varied in depth, surface and catchment area. Among the lakes sited in the plateau, Midge Lake was the deepest, showing a maximum depth during the open-water conditions of 9 m and a surface area of 0.054 Km^2, being furthermore the larger water body of the site. The other lakes located in the platform were shallower, with depths ranging 2.2-5.5 m, excepting lakes Somero and Aså, whose depths were only 0.5 and 0.8 m respectively. The surface of these lakes ranged from 0.039 Km^2 such as Chester Lake to around 0.01 Km^2 as in the case of Lake Somero and Lake Chica. A substantial variation in the water level occurred in some lakes at the thawing period. It is because the outlets were located in some cases in a narrow gorge. During the ice melting, the dams blocking these channels break suddenly, thus producing a substantial decrease of water level in few hours. This occurred, for instance, in Lake Turbio at the night of Dec 18th 2001, when a fast reduction of around 3 m occurred in the water level, from 7.8 to 4.6 m. By contrast, the lakes located in the beaches were consistently shallow, showing depths around or below 0.5 m. They also were somewhat smaller (~0.01 Km^2) compared to those from the plateau. By contrast, the highest catchment areas (>2 Km^2) occurred in some of these coastal lakes such as Lake Maderos and Limnícolas. The extent of catchments in the plateau varied greatly. Thus, Lake Chica or Cerro Negro showed low catchment areas (0.01 Km^2); whereas bigger catchments (>0.5 Km^2) occurred, for instance, in lakes Limnopolar and Turbio.

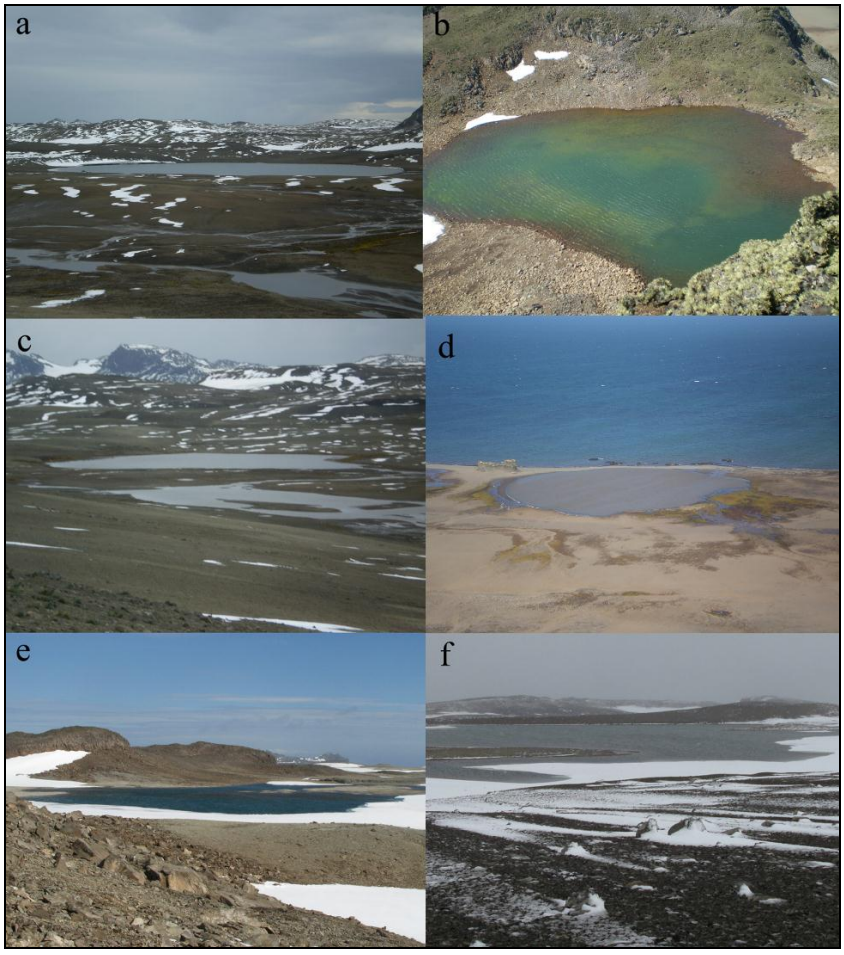

Figure 3.1. *Pictures of some of the lakes studied in Byers Peninsula during summer 2001/02. a)Lake Limnopolar, b) Lake Cerro Negro, c) Lake Somero (upper in the picture), d) Lake Refugio, e) Lake Turbio, f) Chester Cone Lake.*

Table 3.2. *Geographical location and morphometric characteristics of lakes studied during summer 2001/02 in Peninsula Byers. Names assigned to lakes are according to Limnopolar Project.*

Label	Lake	X-UTM	Y-UTM	Catchment size (km^2)	Lake surface (km^2)	Max depth (m)
L1	Limnopolar	597,100	3,052,200	0.58	0.023	5.5
L2	Somero	596,800	3,052,150	0.06	0.011	0.7
L3	Midge Lake	597,700	3,054,150	0.27	0.054	9
L4	Chester Cone	597,500	3,053,550	0.09	0.039	5
L5	Maderos	594,650	3,050,650	2.41	0.010	0.5
L6	Refugio	602,200	3,050,550	0.12	0.016	0.5
L7	Cerro Negro	602,425	3,051,275	0.01	-	2.2
L8	Domo	603,850	3,052,175	0.18	0.029	4.5
L9	Diablo	594,800	3,049,950	0.29	-	-
L10	Limícolas	594,825	3,050,900	2.24	-	0.2
L11	Chica	597,100	3,051,050	0.01	0.008	3.5
L12	Escondido	599,475	3,052,650	0.08	0.022	4.5
L13	Aså	596,975	3,054,100	0.07	0.024	0.8
L14	Las Palmas	602,050	3,052,300	0.12	0.022	2.2
L15	Turbio	598,000	3,051,800	0.58	0.021	7.8

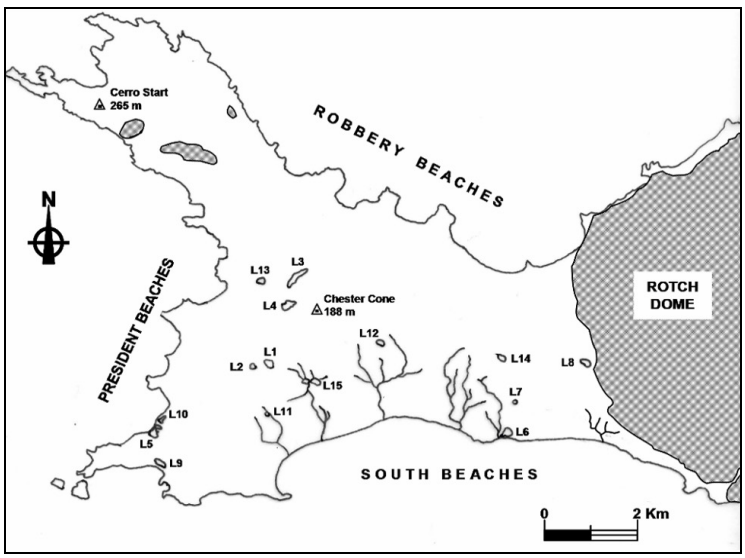

Fig. 3.2. *Map of Byers Peninsula showing the location of lakes studied during summer 2001/02. The labels of the lakes are indicated in table 3.2.*

3.3.3. Ice cover condition of lakes

At the beginning of studied period (December 2001) most of the lakes sited in the plateau were almost ice-covered, They showed a variable ice thickness at this time (Limnopolar, 45 cm; Somero, 30 cm; Midge Lake, 65 cm; Chester Cone, 60 cm), however, a rapid melt-out occurred within the next weeks. At the end of December, the ice covers reduced to about 10–20% of the surface of the most lakes. Still, a delayed melt-out was observed in lakes from the plateau compared to coastal lagoons (1–2 weeks earlier thaw). Nonetheless, a complete melt-out took place in all lakes at the commencement of January, which resulted in observable changes in the physical and chemical structure of water column.

3.3.4. Temperature

The water temperature of lakes increased as season advanced (Fig. 3.3). In general, temperatures ranged from 0.5–2°C in December up to maximum nearly 10°C in February. Lowest temperatures were recorded in the surface of Midge Lake at 19th December when a partial ice-cover remained. By contrast, the higher temperatures occurred in the shallower isothermal lakes such as Refugio (9.8°C) and Somero (11°C). The range of temporal variation was also higher in these shallow lagoons as depicted in figure 3.3 for Lake Somero.

The early lake profiled was Midge Lake on 19th December. At this time, from surface to around 2 m, temperature increased sharply from 0.22ºC to 1.97ºC, though it was nearly constant at 2.40°C between 2 and 6.7 m. Below (until the bottom: 8.25 m), temperature increased sharply until reach temperatures around the higher density (~4 ºC). In other lakes, profiles showed in contrast a temperature distribution nearly homogeneous with depth. Only a slight increase of temperature was observed at 2 m in the profile performed in Lake Turbio at middle January.

3.3.5. Dissolved oxygen

Concerning the dissolved oxygen concentrations, saturated or nearly saturated conditions were generally observed; however, some differences occurred depending of site and period (Fig. 3.4 and 3.5). In the lakes located in the platform, concentrations fluctuated normally between 10 and 14 mg L^{-1}, although higher concentrations were marginally observed at Midge Lake (15.7-18.0 mg L^{-1}) at the commencement of summer, when lake was still ice covered. Only the shallow Lake

Refugio exceptionally displayed sub-saturated conditions (around 75%). It was also observed in Chester Cone Lake at February, which showed lower concentrations of around 8.5 mg L^{-1} and saturations around 70% in the entire profile. On the other hand, no subsurface maxima were observed; nevertheless, some slight peaks were observed occasionally between 1 and 2 m in some lakes during January (i.e. Limnopolar, Turbio, Escondido).

The higher oxygen concentrations were always measured at the beginning of summer, when ice melting was not complete. In the lakes with a higher ice thicknesses (i.e. Lake Chester and Midge Lake), it was observed a continuous increase of oxygen with depth. In the Midge Lake, for instance , both oxygen concentration and saturation mimicked closely the temperature profile at this time. In opposition, a slight decline of the oxygen concentrations with depth was observed in Turbio and Escondido at the early and middle summer.

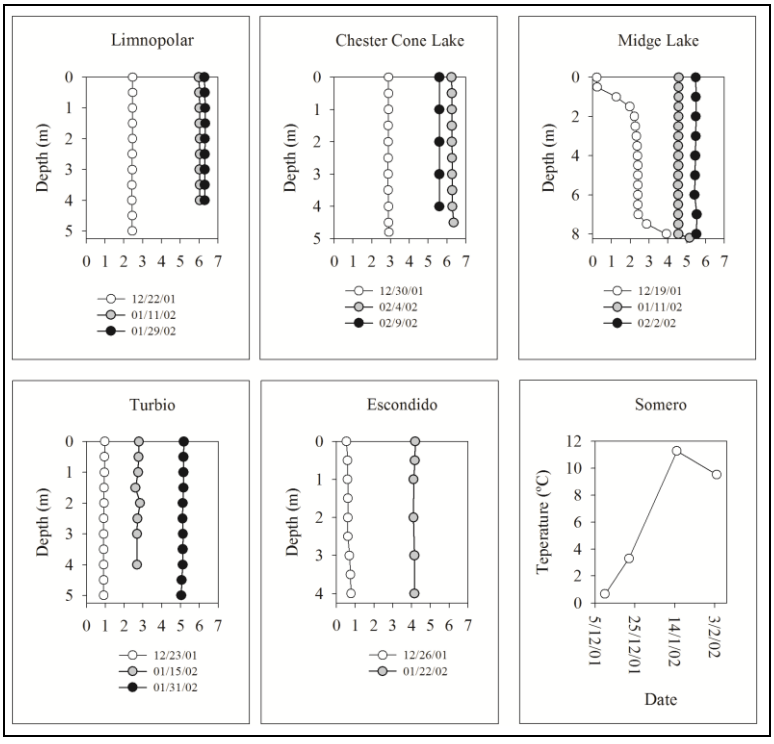

Figure 3.3. *Water-column profiles of temperature (ºC) in the studied lakes during summer 2001/02. Temperature in Lake Somero was measured at 0.1 m depth.*

3.3.6. pH

The pH values in lakes fluctuated around circum-neutral or slightly acidic, showing invariably an increase as period advanced. Thus, the pH values in December and January in the lakes profiled were normally below 7 (Fig. 3.6). A small increase with depth occurred in some lakes (Limopolar, Midge Lake and Turbio) during this period, which contrasted with the observed at final of January and the start of February when surface waters were habitually more alkaline. This regular trend differed particularly in Lake Escondido, where the pH was higher in surface also at December. Also pH values increased with time in the shallow lagoons as observed for Lake Somero (Fig. 3.6); In coastal sites, water bodies exhibited slight higher pH compared to others (normally up to 7.0).

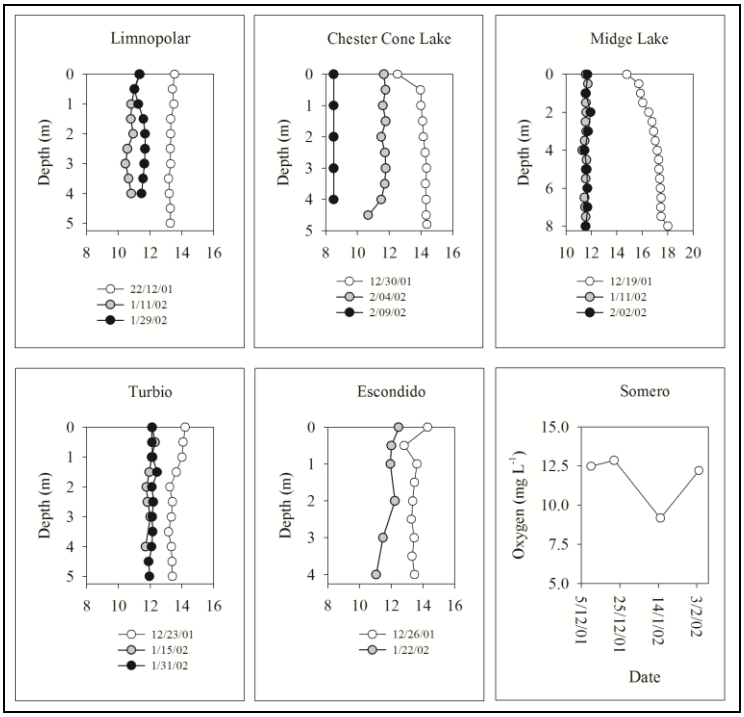

Figure 3.4. *Water-column profiles of oxygen concentration (mg L^{-1}) in the studied lakes during summer 2001/02. Oxygen concentration in Lake Somero was measured at 0.1 m depth.*

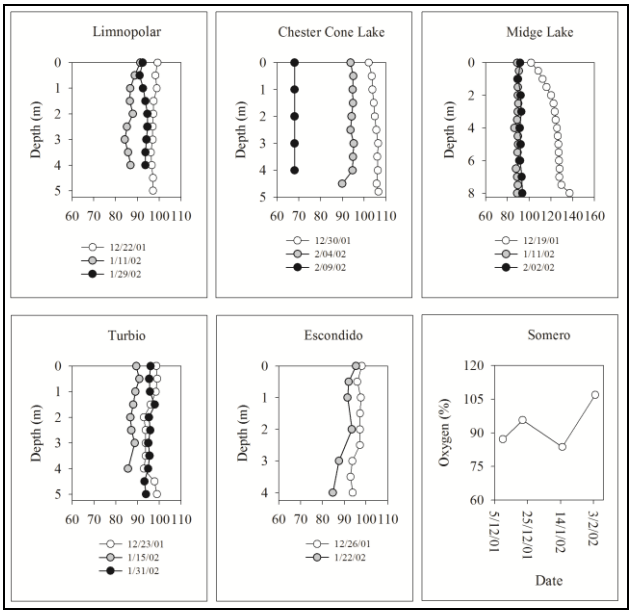

Figure 3.5. *Water-column profiles of oxygen saturation (%) in selected lakes during summer 2001/02. Oxygen saturation in Lake Somero was measured at 0.1 m depth.*

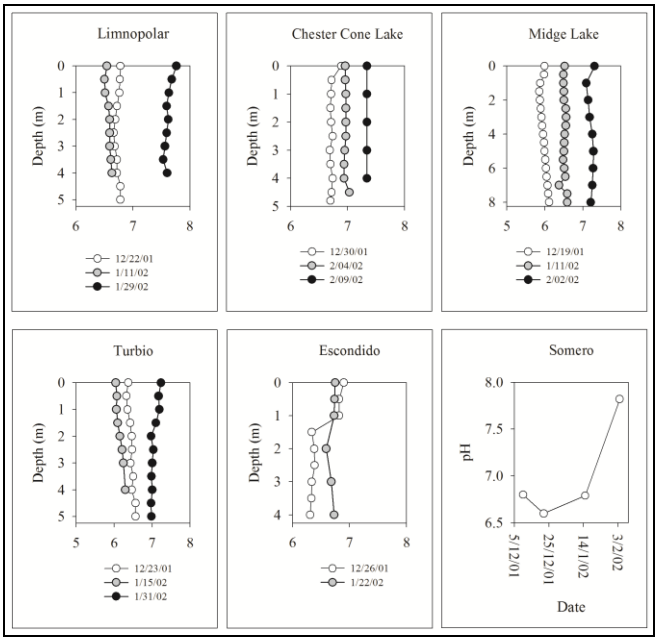

Figure 3.6. *Water-column profiles of pH in the studied lakes during summer 2001/02. The pH in Lake Somero was measured at 0.1 m depth.*

3.3.7. Photosynthetic active radiation profiles

In relation to the underwater PAR profiles (400-700 nm, Fig. 3.7), all the lakes were highly transparent except Lake Turbio. Therefore, around 25% of surface incident light arrived to the bottom of lakes, however, in Lake Turbio, strong light attenuations were measured at December and January, when only 1% of the incident light arrived at 1 m depth. Otherwise, somewhat seasonal variation in PAR penetration occurred in Midge Lake. There, PAR decreases more steeply from surface until around 1 m at middle December, just when ice thaw started. The light reaching the bottom of lake in this case was below 10% of the incident. By contrast, at middle January, the light extinction was almost constant with depth.

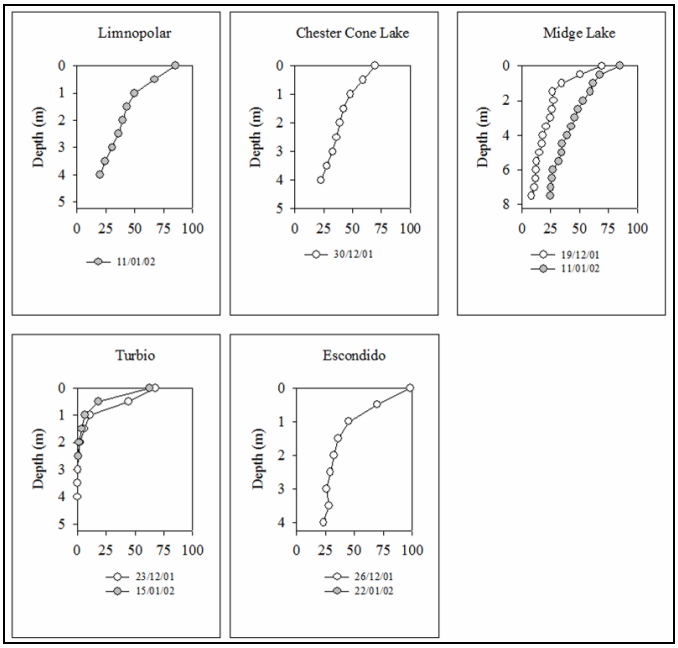

Figure 3.7. *Water-column profiles of the percentage of surface photosynthetic active radiation (PAR) in selected lakes during summer 2001/02.*

3.3.8. Water mineralization

Conductivity was low in general and varied in all lakes from 30 to 189 µS cm^{-1} (Table 3.3). The lower average conductivities (below 50 µS cm^{-1}) were observed in lakes sited far from the sea and closest to the glacier front (Domo, Las Palmas).

Similar values were measured in Lake Cerro Negro and Chica, which are near the sea compared to the former; however, they had closed catchments sheltered from marine inputs. On the other hand, the other lakes located in the central plateau displayed intermediate salinities. In these lakes, values in the entire water column ranged from 52 to 105 µS cm^{-1}, being the higher values registered in the shallowest Lake Somero at February (Fig. 3.8). The higher conductivities, no higher than 200 µS cm^{-1}, occurred in coastal lagoons, with lower and higher values observed in Lake Diablo and Maderos respectively. Concerning the temporal variation, there was not a clear pattern; thus, some lakes showed higher conductivities at the beginning (Midge Lake, Turbio, Escondido); however, a gradual increase with time was observed in others (Limnoplar, Chester, Somero, Refugio).

Among the lakes profiled (Fig. 3.8), the largest vertical gradient was measured in Midge Lake at the survey performed in December. From surface to 1 m, conductivity increased sharply from 38 to 76 µS cm^{-1}. From this depth to around 7 m conductivity kept nearly constant, showing only a small increase close to the sediment (8 m). In general, the other lakes showed more uniform profiles, however, slight increases were observed after the ice melting in the bottom of some lakes (i.e. Turbio, Escondido). By contrast, other lakes such as Limnopolar and Chester showed profiles merely isohalines.

In agree with the low conductivities observed, the content of major ions in lakes was relatively low (Table 3.3). Still, there was a clear distinction between lakes located in the platform, mainly those easternmost, and the harder waters of coastal lagoons. Both Cl^- and Na^+ were the main anion and cation respectively. The highest Cl^- and Na^+ mean concentrations were observed in Lake Maderos (143.00 and 107.20 mg L^{-1} respectively), whereas the mean values in the rest of lakes ranged 8.22-25.70 and 6.41-27.05 mg L^{-1} respectively, being the higher values observed in Lake Refugio. The mean concentrations of Ca^{2+} and Mg^{2+} were also highest in Lake Maderos (16.62 and 11.95 mg L^{-1} respectively). In the other lakes, they ranged 0.42-3.47 mg L^{-1} for Ca^{2+} and 0.03-0.36 mg L^{-1} for Mg^{2+}. Remarkably, the Lake Refugio showed in both cases the lower values of range. The SO_4^{2-} also displayed differences among lakes, with lower and higher values of 1.57 and 31.03 mg L^{-1} in lakes Domo and Maderos respectively. Alkalinity varied in a lower range from 0.01 to 1.21 meq L^{-1}.

The relative proportion of major anions and cations was displayed in ternary diagrams to identify trends in mineral composition of water bodies (Fig 3.9). The data showed are the percentages of their gram-equivalent concentrations, which allow establishing comparisons among them independently of total ionic

concentrations. With regards to cations, Mg^{+2} had always <20% whereas $Na^{+}+K^{+}$ proportions varied greatly in a range between 45% and 96%, being evenly distributed throughout the range. Among lakes, Limnopolar and Refugio showed the minimum and maximum relative content respectively. In the case of Ca^{+2} proportions were also variable but lower, falling between 1.5-35%. Based on it, lakes distributed gradually from a great dominance of $Na^{+}+K^{+}$, as in coastal lagoons and lakes sited eastern (i.e., Escondido, Palmas, Domo), to a major relative abundance of Ca^{+2}, such as in lakes located in the central part of the plateau.

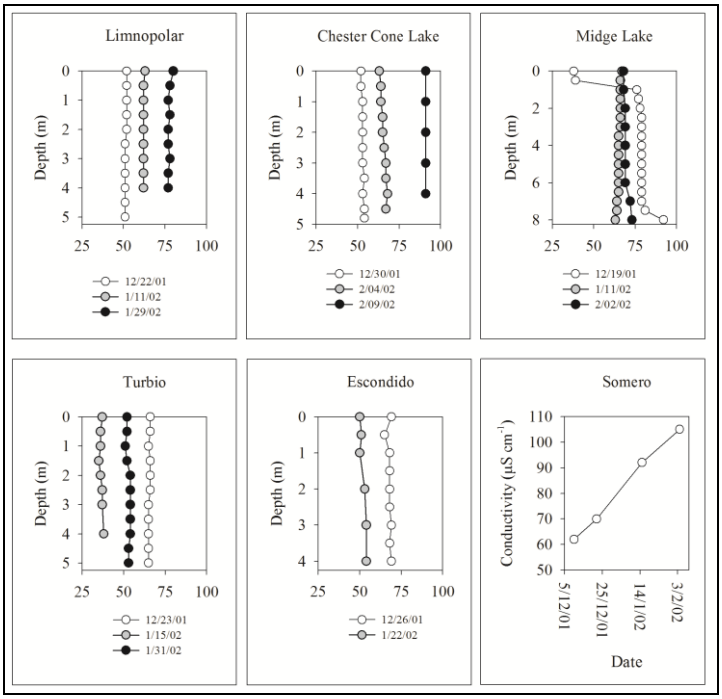

Figure 3.8. *Water-column profiles of conductivity (µS cm^{-1}) in selected lakes during summer 2001/02. Oxygen saturation in Lake Somero was measured at 0.1 m depth.*

Concerning to the anions, a higher variability was observed for Cl^{-} (31.6-75.7%) and $HCO_3^{-}+CO_3^{2-}$ (1.7-64.1%) compared to SO^{4-}, which was always below 23%. Accordingly, lakes divided into two groups, that is, one composed by lakes with Cl^{-} proportions higher than 70% (i.e., Maderos, Aså, Escondido) and other showing a the relative proportion of Cl^{-} between 27-49%. The Gibbs diagram (Gibbs 1970) for the lakes, which is obtained plotting TDS against the weight ratio $[Na^{+}/(Na^{+} + Ca^{2+})]$, is shown in figure 3.10. The data fall inside a boomerang-shaped

envelope (dashed line of figure 3.10). Following the Gibbs idea, which is based on empirical observations, the position of any data in the diagram is largely the balance of processes controlling the chemistry of surface waters, that is, rock weathering, atmospheric precipitation and evaporation/crystallization. In our samples, the weight $Na^+/(Na^+ + Ca^{2+})$ ratio varied between 0.58 and 0.98, showing no covariation with TDS, which ranged 19.8-126.6 ppm. Our data fell within the middle-low part of boomerang envelope. Attending to TDS, data distributed in the water–rock interaction area; however, the relation between sodium and calcium was the expected if the atmospheric precipitation processes were also partially involved. Some data were outside the boomerang, principally those pertaining to the both coastal lagoons computed in the analysis (lakes Maderos and Refugio). Considering the weight ratios of these lakes, they should be positioned in the lower right corner of the boomerang-shaped space.

Table 3.3. *Mean values of conductivities (Cond; µS cm^{-1}) and ions concentrations (meq L^{-1}) measured during summer 2001/02 in lakes of Peninsula Byers.*

Lake	Cond	SO_4^{2-}	Cl^-	Na^+	K^+	Mg^{2+}	Ca^{2+}	Alk
Limnopolar	65.5	0.25	0.46	0.46	0.009	0.21	0.36	0.96
Somero	70.0	0.09	0.74	0.71	0.013	0.29	0.29	0.93
Midge Lake	68.0	0.15	0.46	0.70	0.012	0.21	0.23	0.61
Chester Cone	51.5	0.18	0.39	0.40	0.009	0.14	0.18	0.49
Maderos	189.0	0.65	4.21	4.87	0.107	1.00	0.83	0.60
Refugio	131.0	0.17	0.76	1.23	0.045	0.03	0.02	1.21
Cerro Negro	51.0	-	-	-	-	-	-	-
Domo	29.5	0.03	0.24	0.31	0.006	0.07	0.04	0.49
Diablo	90.0	-	-	-	-	-	-	-
Limícolas	125.0	-	-	-	-	-	-	-
Chica	40.0	-	-	-	-	-	-	-
Escondido	59.5	0.09	0.74	0.64	0.015	0.15	0.07	0.12
Aså	73.0	0.14	0.45	0.50	0.011	0.16	0.19	0.01
Las Palmas	31.5	0.05	0.40	0.44	0.000	0.10	0.07	0.65
Turbio	58.0	0.05	0.26	0.29	0.008	0.09	0.15	0.22

3.3.9. Nutrients concentrations

The concentrations of major inorganic nutrients in the different lakes and in the pond are summarized in table 3.4. Most of these water bodies ranged from ultra-oligotrophic to oligotrophic status, except some located close to the coast, such as Lake Refugio and the pond, in which marine animals like elephant seals supplied high amounts of nutrients. In lakes sited in the plateau, the concentrations of soluble reactive phosphorus (SRP) varied narrowly from levels below detection (<0.03 µM, Lake Chester) to 0.2 µM in Lake Escondido. Among the coastal lagoons, SRP

concentrations were noticeably higher in Lake Refugio (2.9 µM), whereas in Lake Maderos concentrations were similar to those measured in the lakes from the plateau. The highest SRP concentrations (11.3-14.6 µM) were marginally measured in the pond located next to the elephant seals colonies. Remarkable differences were also observed with regards combined nitrogen. Thus, nitrate plus nitrite (NOx) ranged 0.13-1.81 µM and 14.9-25.9 µM in lakes from upland and coastal sites respectively. The NH4 concentrations in lakes located upland ranged 0.64-4.57 µM. In the coastal sites, they were rather similar to the former, except in the pond, in which concentrations were particularly higher (800 µM). Significant differences in silica concentrations occurred in some cases. The lakes located in the central part of the plateau (Lakes Domo, Las Palmas and Escondido) displayed very low silica concentrations (3.4-8.9 µM). By contrast, in the rest (including coastal lagoons) the concentrations varied in a higher range between 21.3 and 130 µM, being the highest measured in the shallower water bodies (Somero, Refugio and in the pond).

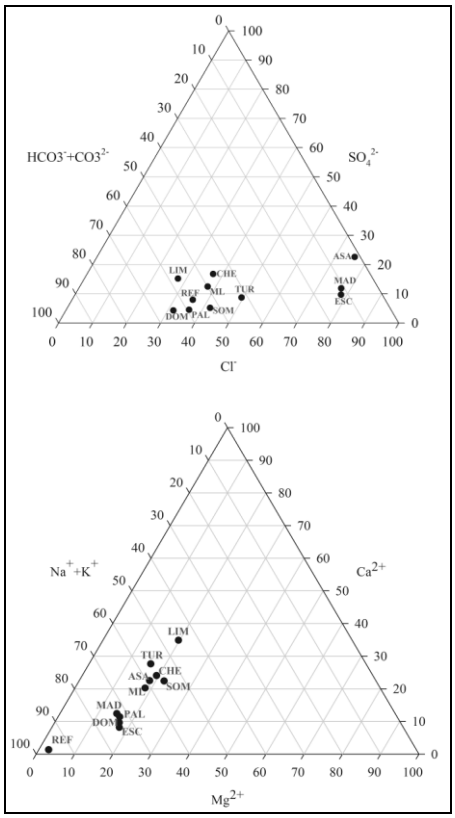

Figure 3.9. *Ternary plots showing the percentage of cations and anions in lakes from Byers Peninsula. Proportions are based on data in meq L^{-1}. Lake Aså (ASA); Lake Chester Cone (CHE); Lake Chica (CHI); Lake Domo (DOM); Lake Escondido (ESC); Lake Limnopolar (LIM); Lake Maderos (MAD); Midge Lake (MIL); Lake Las Palmas (PAL); Lake Refugio (REF); Lake Somero (SOM); Lake Turbio (TUR).*

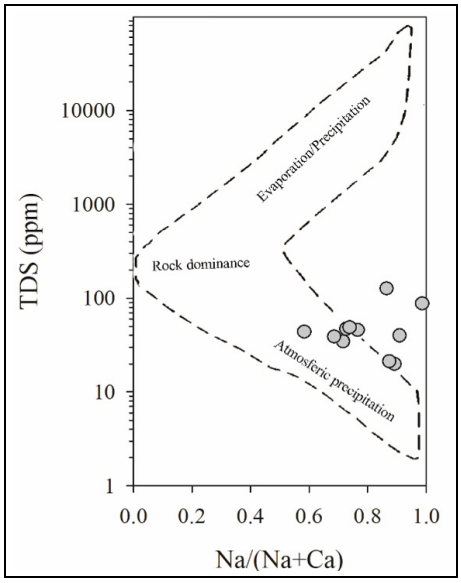

Figure 3.10. *Plot showing the position in the Gibbs model of surface waters of lakes from Byers Peninsula. See text for details.*

With the exception of the pond, whose values were approximately 100-folds higher to the more oligotrophic lakes; total nitrogen (TN) and total phosphorus (TP) in ranged 4.00-171.18 µM and 0.09-21.05 respectively. Among them, the coastal lake Refugio displayed the highest concentrations followed by Lake Maderos. Contrastingly, the concentrations in the lakes from the plateau were approximately one order of magnitude lower, being below 25 and 2 µM of TN and TP respectively. The TN/TP relationships varied also notably between lakes showing relatively balanced ratios (i.e., Refugio, Turbio, Somero, Aså, and Maderos) to those displaying a notable P-deficiency such as Lake Escondido and particularly in Lake Chester and Midge Lake. The concentrations of dissolved organic carbon (DOC) were occasionally measured in some lakes. The values ranged 0.5-3.5 mg L^{-1}, being the highest and lowest observed in Lake Refugio and Midge Lake respectively. In general, DOC concentrations increased as the lake's trophic status.

3.3.10. Photosynthetic pigments

The chlorophyll-a (Chl-a) concentrations in lakes sited in the plateau were consistently lower compared to coastal sites (Table 3.5). In the former, the concentrations varied from 0.06 to 2.22 µg L^{-1}. In plateau lakes, the lower values (<0.1 µg L-1) were recorded in December in the surface waters of some lakes

located easternmost (Las Palmas and Domo) as well in the surface of lake Chester and at 4 m in Midge Lake. Among the other lakes sited in the plateau, Lake Escondido and those located in its central part, concentrations differed depending of lake depth. Thus, in mid-shallow lakes (Limnopolar, Turbio and Escondido) concentrations ranged around 0.1-0.5, whereas in shallower lakes (Somero, Chica and Aså), they were somewhat higher, varying between 0.7 and 2.22 μg L^{-1}.

Table 3.4. *Nutrient concentrations measured during the survey period (summer 2001/02) in water bodies of Byers Peninsula. All concentrations are expressed in μM except DOC which are expressed in mg L^{-1}.*

Lake	Sampling Date	Sampling depth (m)	NOx	NH_4	SRP	SRSi	TN	TP	TN/TP	DOC
Domo	26/12/01	2	0.35	1.46	0.06	4.3	13.21	0.55	24.0	-
Domo	06/02/02	0.5	0.46	3.35	0.13	13.5	23.95	0.43	55.7	0.6
Escondido	26/12/01	2	9.39	1.34	0.22	4.0	19.80	0.24	82.5	-
Escondido	22/01/02	0.5	0.45	1.74	0.18	2.8	14.71	0.31	47.5	-
Las Palmas	26/12/01	0.5	0.52	2.11	0.06	4.5	4.00	0.09	44.4	-
Limnopolar	22/12/01	2.5	0.17	4.20	0.09	25.2	11.6	0.52	22.3	-
Limnopolar	11/01/02	2	0.32	7.38	0.03	49.2	16.06	0.38	42.3	-
Limnopolar	29/01/02	0.5	0.11	2.15	0.06	64.9	12.03	0.13	92.5	1
Somero	22/12/01	0.1	0.21	2.96	0.03	62.7	23.26	1.48	15.7	-
Somero	11/01/02	0.1	0.17	3.04	0.07	68.3	6.83	1.66	4.1	-
Somero	04/02/02	0.1	0.09	1.07	0.04	85.3	14.82	0.57	26.0	1.7
Midge Lake	19/12/01	0.5	2.81	2.53	<0.03	45.9	14.28	0.28	51.0	-
Midge Lake	19/12/01	4	1.48	1.99	0.05	51.7	18.88	0.07	269.7	-
Midge Lake	11/01/02	4	0.15	<0.5	<0.03	49.2	23.48	0.13	180.6	-
Midge Lake	02/02/02	0.5	0.27	2.39	0.14	50.1	15.54	0.61	25.5	0.5
Chester	30/12/01	2.5	1.16	0.50	0.04	21.1	10.79	0.11	98.1	-
Chester	04/02/02	0.5	0.27	2.58	<0.03	41.7	11.22	0.63	17.8	1.1
Turbio	23/12/01	2.5	0.98	0.73	0.04	12.2	17.13	1.27	13.5	-
Turbio	15/01/02	0.5	0.40	0.86	0.08	24.0	18.2	1.49	12.2	-
Turbio	31/01/02	0.5	0.36	1.19	0.07	35.6	7.95	0.49	16.2	0.5
Aså	30/12/01	0.5	1.82	0.64	0.11	32.2	12.00	1.21	9.9	-
Chica	07/02/02	0.5	0.13	2.38	0.05	46.0	6.5	1.27	5.1	0.6
Maderos	21/12/01	0.1	14.96	1.67	0.24	21.3	112.2	11.2	10.0	-
Refugio	21/01/02	0.1	48.02	2.19	2.70	71.6	171.18	21.5	8.0	-
Refugio	06/02/02	0.1	3.84	2.29	3.11	-	91.30	15.3	6.0	3.5
Pond	21/01/02	0.1	23.55	860	11.34	130.0	1,236	21	58.9	-

Tabla 3.5. *Concentrations (µg L^{-1}) of clorophyll-a (Chl-a) and relative amounts (µg µg Chl-a^{-1}) of two predominant taxa-specific carotenoids measured in lakes from Peninsula Byers during summer 2001/02.*

Lake	Date	Depth (m)	Chl-*a*	Lutein	Fucoxanthin
Domo	26/12/2001	2	0.074	0.018	0.061
Escondido	26/12/2001	2	0.250	0.039	0.042
Escondido	22/01/2002	0.5	0.268	0.102	0.052
Las Palmas	26/12/2001	0.5	0.071	0.060	0.049
Limnopolar	22/12/2001	2.5	0.137	0.048	0.047
Limnopolar	11/01/2002	2	0.181	0.125	0.129
Limnopolar	29/01/2002	0.5	0.133	0.218	0.227
Somero	22/12/2001	0.1	2.220	0.152	0.312
Somero	11/01/2002	0.1	0.758	0.157	0.023
Somero	04/02/2002	0.1	0.789	0.303	0.014
Midge Lake	19/12/2001	4	0.078	0.105	0.049
Midge Lake	11/01/2002	4	0.152	0.085	0.090
Midge Lake	02/02/2002	0.5	0.154	0.154	0.086
Chester	30/12/2001	2.5	0.066	0.012	0.079
Turbio	23/12/2001	2.5	0.465	0.076	0.037
Turbio	15/01/2002	0.5	0.578	0.097	0.031
Turbio	31/01/2002	0.5	0.523	0.107	0.031
Aså	30/12/2001	0.5	1.273	0.162	0.018
Chica	07/02/2002	0.5	0.811	0.262	n.d.
Maderos	21/12/2001	0.1	1.445	0.220	0.088
Refugio	21/01/2002	0.1	40.51	0.139	n.d.
Refugio	06/02/2002	0.1	15.30	0.150	n.d.
Pond	21/01/2002	0.1	54.94	0.052	n.d.

In some of the coastal sites Chl-a concentrations were at least 10-folds higher and variable with time. In Lake Refugio for instance, the Chl-a concentration decreased with time from 40.5 to 17.0 µg L^{-1}. A discrete sampling in the pond showed a concentration as high as 54.9 µg L^{-1}. The Chl-a concentration recorded in Lake Maderos was by contrast in the order of the observed for upland lakes. The HPLC chromatograms extracted at 440 nm of some of the lakes studied are shown in figure 3.11, demonstrating the elution order of the different photosynthetic pigments. Trends in phytoplankton community structure can be partially derived from the

relative concentrations (i,e., relative to Chl-a) of the predominant taxa-specific carotenoids, which are summarized in table 3.5. They shows a major presence of specific carotenoids of some algal groups such as chlorophyceae (lutein, violaxanthin, neoxanthin, clorophyll-b), and both chrysophyceae and diatoms (fucoxanthin, diadinoxanthin, clorophyll-c).

With regards to lutein, a characteristic carotenoid of chlorophytes, relative amounts ranged in all lakes from 0.01 to 0.30. As a rule, they increased with time, in this sense, the lowest values (<0.07) were observed in December in lakes situated at the eastern part of the Peninsula as well in some from the central part (i.e. Lake Chester). In the other lakes located upland values were between 0.08 and 0.30, with the lowest values also at the beginning of summer. The relative amount of lutein in coastal sites was similar or even lower (range 0.52-0.26) that those observed in shallowest lakes of the platform (i.e., Somero or Aså). Concerning the fucoxanthin contribution to algal pigment pool (i.e., diatoms and chrysophytes), there was not a clear seasonal trend as observed for lutein. The range of variation among lakes was in this case 0.01-0.31. Nevertheless, with the highest values found in Lake Somero in December (0.31) and in Lake Limnopolar at the end of January (0.17), being in general the values consistently below 0.1.

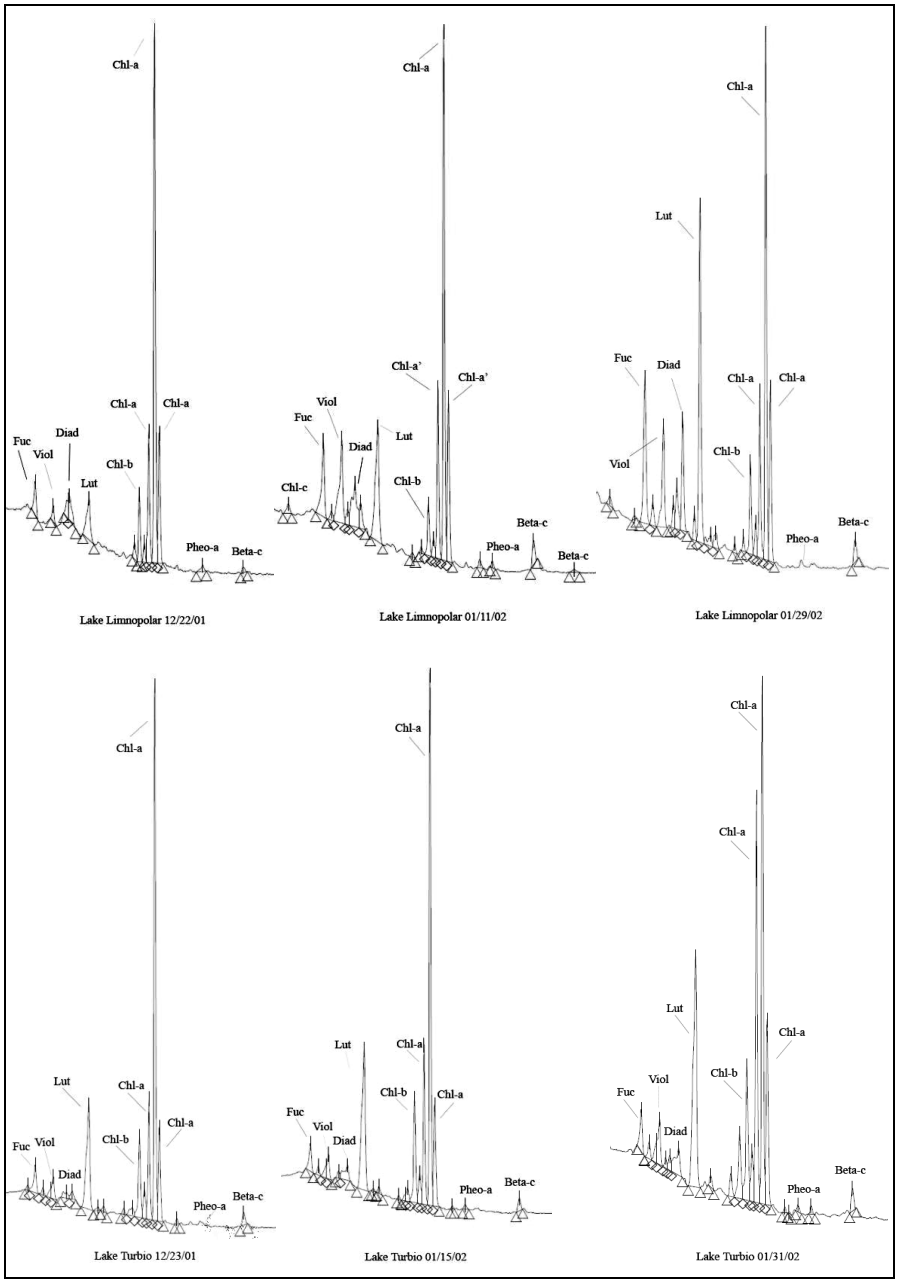

Figure 3.11. *HPLC chromatograms extracted at 440nm showing the photosynthetic pigment composition in some lakes from Peninsula Byers. Fuc=fucoxanthin, Viol=violaxanthin, Diad=diadinoxanthin, Lute=lutein, Chl-b=chlorophyll-b, Chl-b'=chlorophyll-b allomer, Chl-a'= chlorophyll-a allomer, Chl-a= chlorophyll-a, Pheo-a=Phaeophytin-a, Beta-c=β-caroten. The chromatograms are normalized to the same size based on the largest peak (Chl-a) in each of them.*

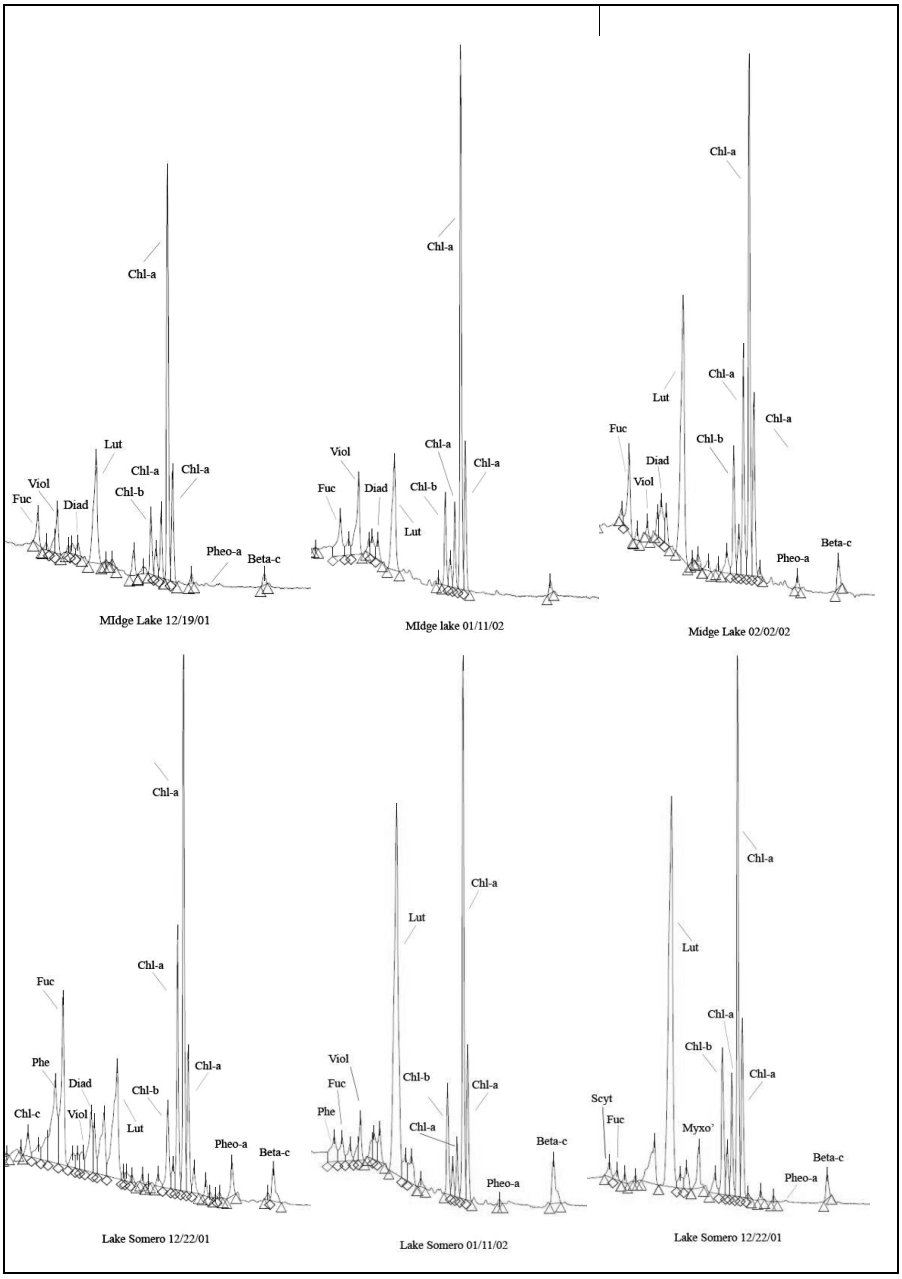

Figure 3.11 (continuation). *HPLC chromatograms extracted at 440nm showing the photosynthetic pigment composition in some lakes from Peninsula Byers. Fuc=fucoxanthin, Viol=violaxanthin, Diad=diadinoxanthin, Lute=lutein, Chl-b=chlorophyll-b, Chl-b'=chlorophyll-b allomer, Chl-a'= chlorophyll-a allomer, Chl-a= chlorophyll-a, Pheo-a=Phaeophytin-a, Beta-c=β-caroten. The chromatograms are normalized to the same size based on the largest peak (Chl-a) in each of them.*

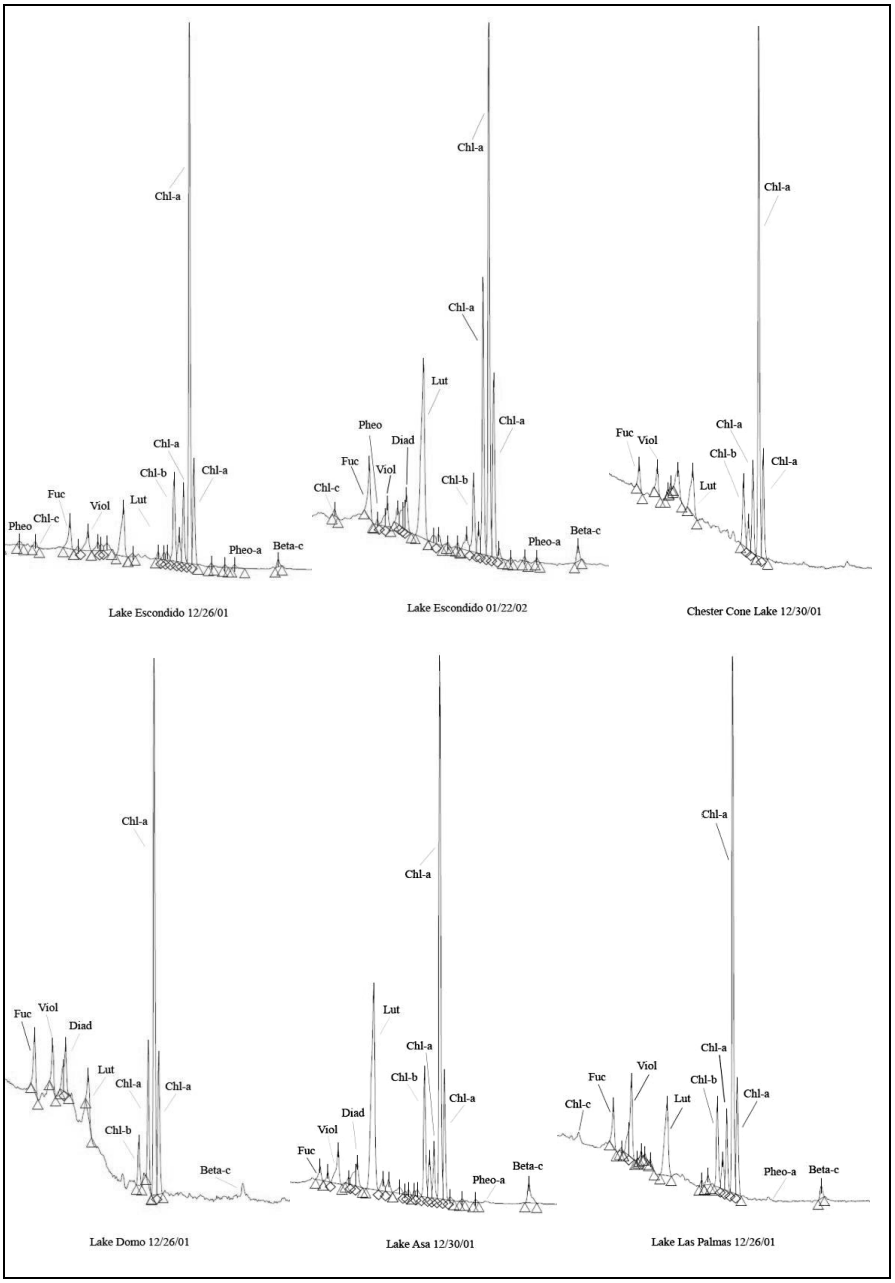

Figure 3.11 (continuation). *HPLC chromatograms extracted at 440nm showing the photosynthetic pigment composition in some lakes from Peninsula Byers. Fuc=fucoxanthin, Viol=violaxanthin, Diad=diadinoxanthin, Lute=lutein, Chl-b=chlorophyll-b, Chl-b'=chlorophyll-b allomer, Chl-a'= chlorophyll-a allomer, Chl-a= chlorophyll-a, Pheo-a=Phaeophytin-a, Beta-c=β-caroten. The chromatograms are normalized to the same size based on the largest peak (Chl-a) in each of them.*

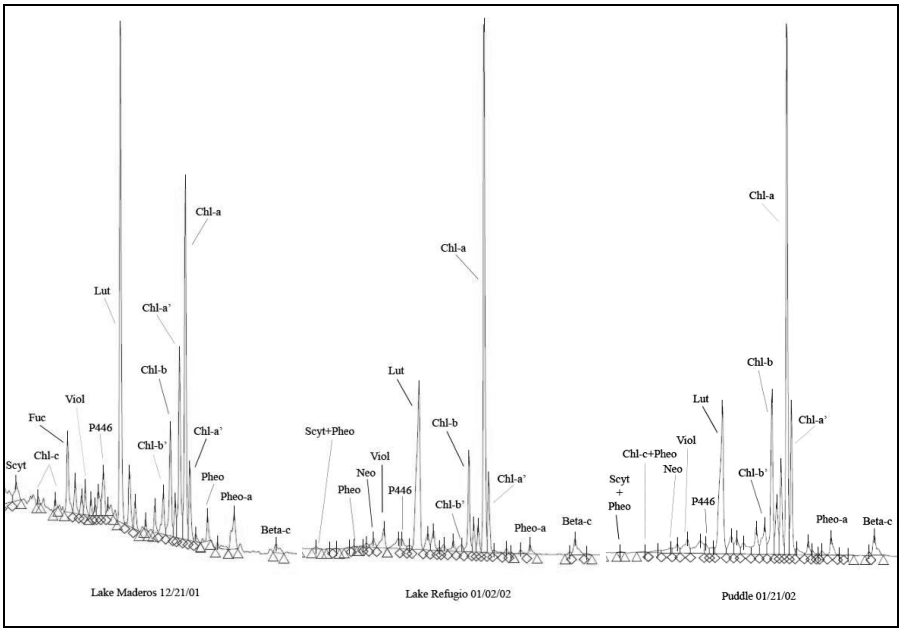

Figure 3.12 (continuation). *HPLC chromatograms extracted at 440nm showing the photosynthetic pigment composition in some lakes from Peninsula Byers. Fuc=fucoxanthin, Viol=violaxanthin, Diad=diadinoxanthin, Lute=lutein, Chl-b=chlorophyll-b, Chl-b'=chlorophyll-b allomer, Chl-a'= chlorophyll-a allomer, Chl-a= chlorophyll-a, Pheo-a=Phaeophytin-a, Beta-c=β-caroten. The chromatograms are normalized to the same size based on the largest peak (Chl-a) in each of them.*

3.3.11. Heterotrophic picoplankton

The abundance of bacterioplankton (=heterotrophic picoplankton, HPP) varied broadly in lakes from 0.5 to 6.5x10^6 cell mL^{-1} (Table 3.6), showing a clear dissimilarity between inland and coastal waterbodies. Thus, bacterial numbers in lakes from the plateau were usually below 1.5x10^6 cell mL^{-1}, except in December just after the complete ice thaw, when some of them displayed higher abundances around 2x10^6 cell mL^{-1} (i.e. Limnopolar, Escondido). Among these lakes, only Lake Somero showed a clear trend of increasing abundances during summer, doubling its bacterial numbers from 1.9x10^6 cell mL^{-1} recorded in December to 4.1x10^6 cell mL^{-1} at the beginning of February. Abundances in coastal water bodies were always higher, being between 3.7x10^6 cell mL^{-1} and 6.6x10^6 cell mL^{-1} in lagoons and even somewhat higher (9.3x10^6 cell mL^{-1}) in the pond.

The distribution of bacteria against depth in those lakes profiled is shown in the figure 3.12. In lakes where higher numbers were measured (~1.7-2 x 10^6 cell

mL^{-1}) these occurred at deep, as observed for lakes Limnopolar and Turbio in middle January, as well as in Lake Escondido in December. On the contrary, the higher densities in lakes Chester Cone and Midge were recorded in surface layers. Differently, Lake Domo showed a quite homogeneous profile at the final of December. A different temporal trend in this lake compared to the others was not confirmed, however, because the lack of subsequent samplings.

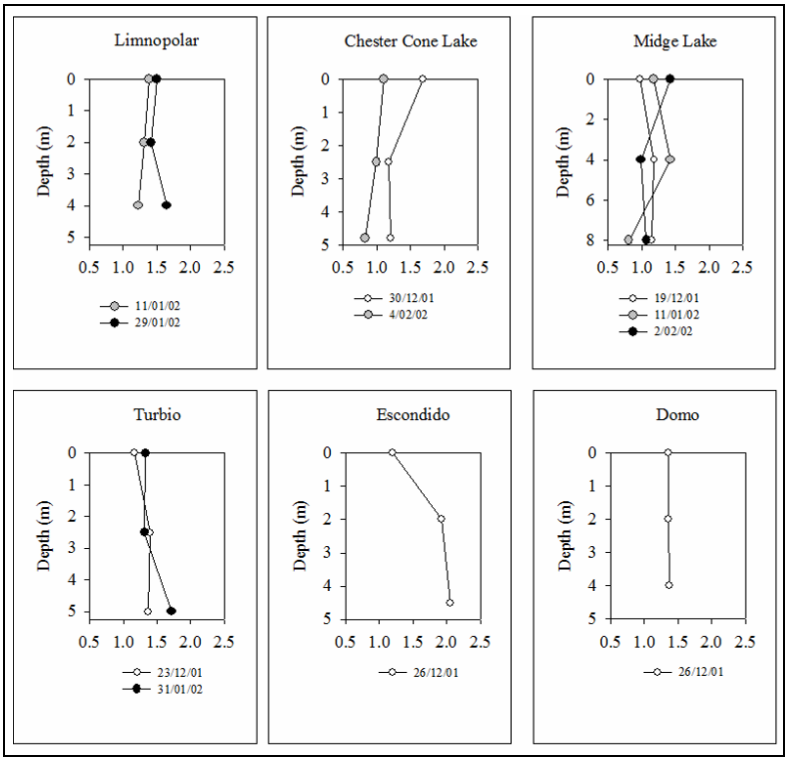

Figure 3.12. *Bacterioplankton (HPP) abundances in the water column of some of the lakes from Peninsula Byers surveyed at summer 2001/02.*

3.3.12. Autotrophic picoplankton

Autotrophic picoplankters were present in all lakes and included both picocyanobacteria (APC; Fig. 3.13a and 3.13b) and small (3-5 µm) unclassified autotrophic picoeukaryotes (APE), most of them flagellates with a cell morphology resembling picoprasinophytes (Fig. 3.13c and 3.13d). In relative terms, eukaryotic forms usually dominated (Table 3.6). APC commonly occurred at low densities

(total range 23-889 cells mL^{-1}). Among lakes located in the plateau, the highest APC abundance was recorded in Lake Chester Cone. Their vertical distribution in this lake changed seasonally from higher numbers in surface waters in December and in deep waters in February. Among the coastal sites, a low abundance was observed in all lagoons (>200 cells mL^{-1}), whereas in the pond densities of $1.85x10^3$ cells mL^{-1} were recorded. The abundances of APE were higher and more variable among lakes. They ranged broadly between 9 to $711x10^3$ cells mL^{-1} in the total range of the lakes studied. Densities in lakes located in the plateau were, nevertheless, always below $6x10^3$ cells mL^{-1}. In the later, abundances peaked normally at surface layers such as in the case of Midge Lake.

3.3.13. Nanoplanktonic protists

The nanoplankton community was composed by a small number of flagellates species (both colourless and plastidic forms), small amoebae and ciliates. With regards to the total abundances of flagellates (Table 3.6), it was moderately low in lakes located upland, in which numbers ranged $0.82\text{-}1.46x10^3$ cell mL^{-1}. Higher densities were recorded, by contrast, in coastal lagoons such as in Lake Maderos ($529x10^3$ cell mL^{-1}), and particularly in Lake Refugio at the beginning of Febraury ($1{,}277x10^3$ cell mL^{-1}). Among them, plastidic forms (PNF) generally dominated over the colourless (HNF). Forms of 3-5 µm long dominated the heterotrophic subset and were assigned to chrysomonads (*Spumella* and *Oikomonas;* Fig. 3.14a). Other fraction of the community belonged to small plastidic flagellates tentatively identified as chrysophytes from genera *Ochromonas*-like and *Chromulina* sp.; however, others could be prasinophytes species. Among the pigmented forms, the more recognizable were chrysophytes composed mainly by two genera of *Pseudokephyrion*, which were especially abundant. With regards to the size structure, they were mainly composed by flagellates sizing 3-6 µm, which contrasted with the higher occurrence of larger cells (10-15 µm) in coastal lagoons. Cryptophytes were also present, but they occurred in very small numbers during the studied period.

Table 3.6. *Abundances (cells mL^{-1}) of pico-sized organisms and nanoflagellates in lakes from Peninsula Byers at summer 2001/02. HPP= heterotrophic picoplankton, APC= picocyanobacteria, APE= autotrophic picoeukaryontes, NF= nanoflagellates.*

Lake	Date	Depth (m)	HPP	APC	APE	NF
Domo	26/12/01	2	1.36 x10^6	41	9	82
Domo	06/02/02	0.5	1.09 x10^6	143	405	278
Escondido	26/12/01	2	1.90 x10^6	239	256	237
Escondido	22/01/02	0.5	1.09 x10^6	128	73	1,059
Las Palmas	26/12/01	0.5	0.70 x10^6	61	1,346	260
Las Palmas	26/12/01	2	1.40 x10^6	34	170	-
Limnopolar	22/12/01	2.5	2.16 x10^6	129	33	82
Limnopolar	11/01/02	2	1.31 x10^6	49	107	304
Limnopolar	29/01/02	0.5	1.50 x10^6	91	90	378
Somero	22/12/01	0.25	1.89 x10^6	98	466	150
Somero	11/01/02	0.25	2.58 x10^6	61	41	832
Somero	04/02/02	0.25	4.06 x10^6	27	164	1,396
Midge Lake	19/12/01	0.5	0.96 x10^6	60	1,404	284
Midge Lake	19/12/01	4	1.18 x10^6	71	1,972	428
Midge Lake	19/12/01	8	1.15 x10^6	57	55	74
Midge Lake	11/01/02	0.5	1.18 x10^6	166	44	106
Midge Lake	11/01/02	4	1.43 x10^6	23	1,479	284
Midge Lake	11/01/02	8	0.81 x10^6	-	-	-
Midge Lake	02/02/02	0.5	1.42 x10^6	93	887	131
Midge Lake	02/02/02	4	0.99 x10^6	-	-	-
Midge Lake	02/02/02	8	1.07 x10^6	-	-	-
Chester Cone	30/12/01	0.5	1.68 x10^6	762	6032	489
Chester Cone	30/12/01	2.5	1.18 x10^6	776	101	71
Chester Cone	30/12/01	4.5	1.20 x10^6	365	4526	62
Chester Cone	04/02/02	0.5	1.11 x10^6	153	n.d.	-
Chester Cone	04/02/02	2	0.99 x10^6	245	n.d.	-
Chester Cone	04/02/02	4	0.83 x10^6	889	258	80
Turbio	23/12/01	2.5	1.40 x10^6	37	45	179
Turbio	15/01/02	0.5	0.86 x10^6	61	-	-
Turbio	31/01/02	0.5	1.33 x10^6	45	173	466
Aså	30/12/01	0.5	1.24 x10^6	75	1,690	1,174
Chica	07/02/02	0.5	1.67 x10^6	69	164	1,461
Maderos	21/12/01	0.25	3.72 x10^6	245	n.d	529,971
Refugio	21/01/02	0.25	6.58 x10^6	299	250,606	686,086
Refugio	06/02/02	0.25	6.04 x10^6	204	710,736	1,277,681
Pond	21/01/02	0.1	9.28 x10^6	1,850	1,972	108,952

Concerning to ciliated protozoa, they were mainly composed by nanoplanktonic species (Fig. 3.14b and c), principally by the small prostomatids *Balanion planktonicum* and *Cyclidium* sp., being the former dominant. The total densities of ciliates were only measured in Lake Limnopolar, where numbers ranged from 640 to 2,790 ind L^{-1}, showing an increase as season advanced. Other ciliates

morphologies were observed in samples but their numbers were far lower than the former. On the other hand, epibiont forms of a peritrich specie resembling *Vorticella* was seldom found profusely attached to copepods (Fig. 3.15b); in any case, we fail to know if this specie alternate sessile and free swimming motile phases.

3.3.14. Metazoan

The diversity of metazoan was very low in all the lakes studied. Copepods were the most important in terms of biomass, and were solely represented by the calanoid species *Boeckella poppei* (Fig. 3.15a). This species was ubiquitous in lakes and occurred in all sampled dates. Counts performed in Lake Limnopolar displayed low abundances (~1 ind L^{-1}). Changes observed during the studied period in age structure of populations were rather coherent among lakes (Fig. 3.16). Therefore, a directional change was observed from a dominance of immature states at the beginning, with the highest percentages of nauplii (3-16%) and copepodites I-III (24-50%) observed in December, to a major domain of mature forms at the final of January. Accordingly, considering all lakes, a significant increase (R^2=0.65; p=0.001; n=14) was observed in the relative contribution of adult stages (stage VI) as summer advanced (Fig. 3.17).

The abundance of pelagic rotifers in general was found to be low (<0.3 ind L^{-1}), and consequently their ecological impact on plankton community can be considered to be minor. The only planktonic species observed in samples was *Notholca* cf. *walterkostei*. On the other hand, more related to benthic habitats especially in shallower lakes, was common the fairy shrimp *Branchinecta gaini* (Fig. 3.15c). This anostraca occurred in high numbers in lakes Somero and Maderos, peaking at middle and late summer. On the other hand, some individuals of the cladoceran *Macrothrix oviformis* (after KOTOV 2007) were also found, but clearly associated to the bottom as a nekto-benthic species, concretely at lakes Limnopolar and Chester Cone. Also in lakes with a well-developed benthos, was noted the presence of two macroinvertebrates, the oligochaete *Lumbricillus* sp. and the chironomid *Parochlus steinenii*, which occurred closely associated to the aquatic mosses.

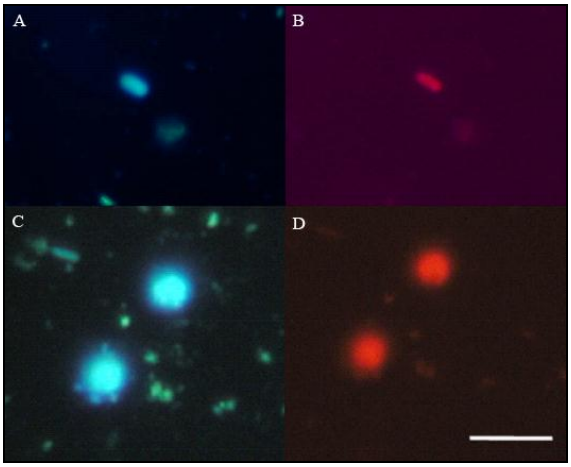

Figure 3.13. *Epifluorescent image of DAPI stained samples showing a pycocyanobacteria (A and B) and a picoprasinophyte-like form (C and D). Pictures A and C were performed with UV light excitation to make out the DAPI staining. The pictures B and D were performed with green light excitation to show chlorophyll-a fluorescence as red. The scale bar indicates 5 µm for all pictures.*

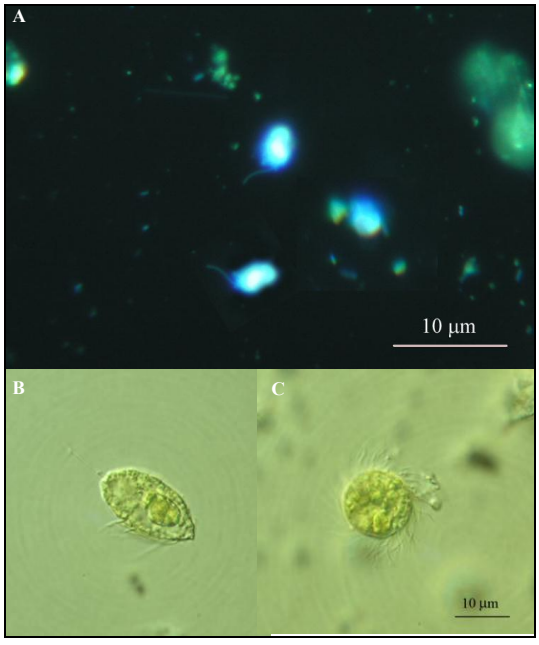

Figure 3.14. *A) Epifluorescence photography showing two specimens of chrysomonad flagellates typically observed in samples from Lake Limnopolar. B and C) Photomicrographs of the two dominated euplanktonik ciliates observed in lakes. The scale bar indicates 10 µm.*

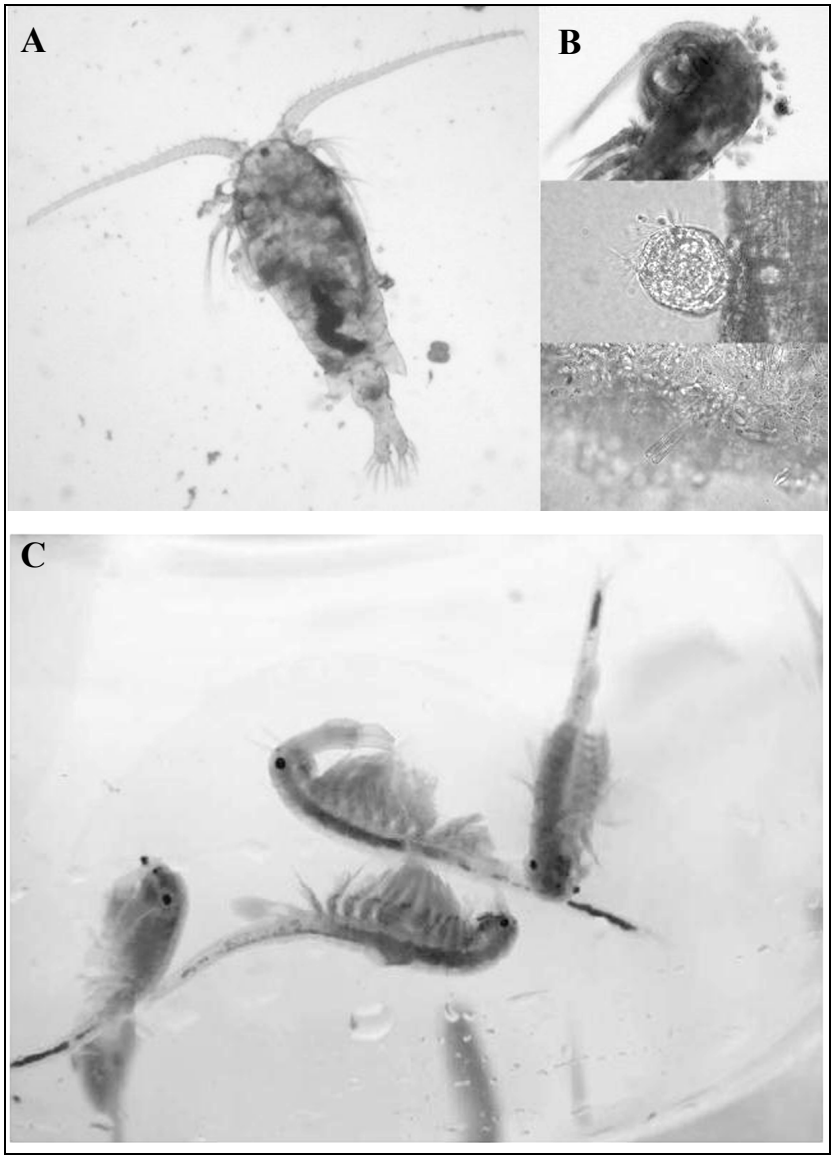

Figure 3.15. *Microscope image showing two major metazoa observed in lakes from Peninsula Byers A) Adult of copepod Boeckella poppei B) Pictures showing epibiont ciliates and diatoms attached to surface of Boeckella poppei. c) Adults of the fairy shrimp Branchinecta gaini.*

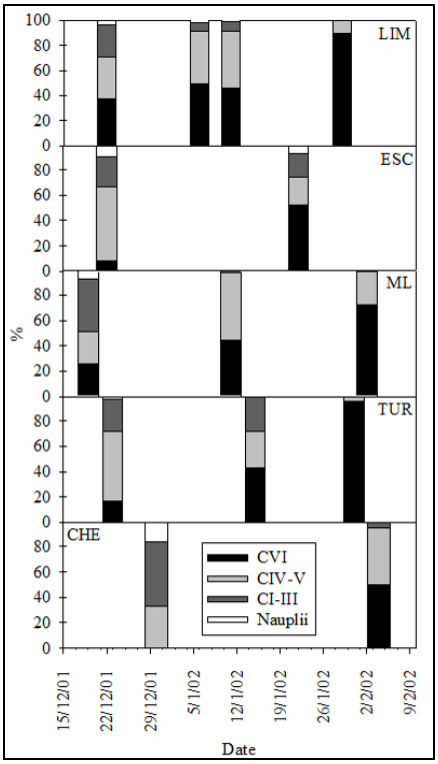

Figure 3.16. *Evolution of the age structure of Boeckella popeii populations in different lakes from Byers Peninsula sampled during summer 2001/02.*

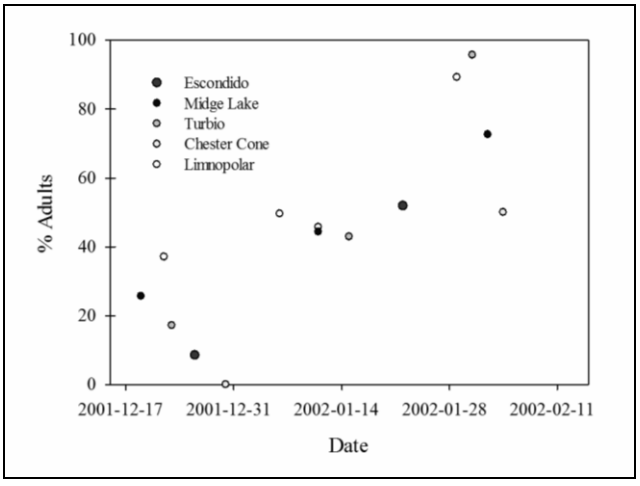

Figure 3.17. *Temporal evolution in the relative contribution of mature stages of Boeckella popeii to the entire population in different lakes during summer 2001/02.*

3.3.15. Benthic flora

Lakes varied in relation to the benthic communities that they supported. The lakes with a well-developed benthic carpet of mosses were Limnopolar (Fig. 3.18), Midge and Chester Cone Lake. In other lakes such as Cerro Negro, Escondido and Aså, small moss patches were found at the bottom or close to the lake shore. They were composed solely by the species *Drepanocladus longifolius*. In addition, both benthic diatoms and filamentous desmidiaceae occurred also as epiphyton on these aquatic mosses. The shallower lakes were in some cases overlaid by thin microbial mats and/or by less coherent biofilms (Fig. 3.19, see chapters 7-9 for a further description). The main constituents of these communities were non-heterocystous filamentous cyanobacteria identiffed as species of Oscillatoriales corresponding to the genera *Phormidium*, *Oscillatoria*, *Lyngbya* and *Leptolyngbya*, with large differences in their relative abundance and mat structure between locations (see chapter 7 for a further description). Diatoms were abundant as well in these benthic communities.

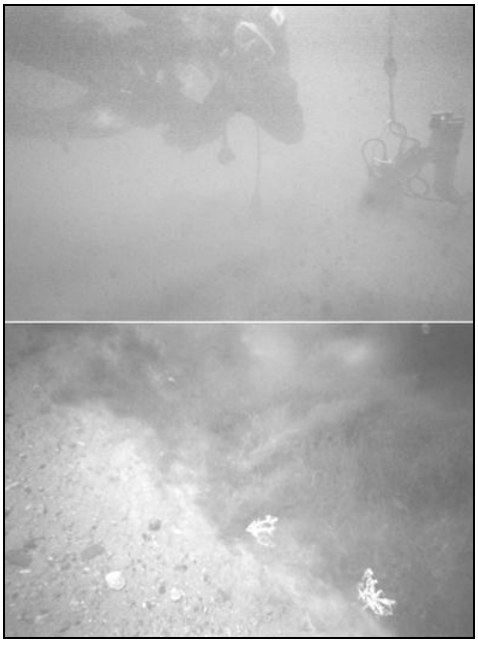

Figure 3.18. *Pictures from the bottom of Lake Limnopolar showing the coverage of mosses (Drepanocladus longifolius. Pictures provided by Javier Cristobo and Dan Dietrich.*

Figure 3.19. *Microbial mats in the catchment of Lake Somero. They thrive also surrounding the shoreline of the lake.*

3.3.16. Multivariate analysis

Principal Component Analyses (PCA) were performed to explore the occurrence of patterns influencing lake characteristics. For the analysis we used only those lakes for which we had a more detailed data set, namely, Limnopolar, Somero, Chester, Midge Lake, Domo, Escondido, Chica, Turbio, Las Palmas, Aså, Refugio, and Maderos. Two different PCA's were run with a different set of cases in order to better evaluate some latent trends. One of them (PCA-1; Fig. 3.20) included data from all the above mentioned lakes, whereas the second (PCA-2; Fig. 3.21) was performed with data from all lakes except lake Maderos and Refugio. In the PCA-1, the first and second axes jointly explained 78.8% of data variability. The analysis clearly differentiated lakes sited in the plateau from those closest to sea (Lakes Maderos and Refugio). The first axis accounted for 45.13% of variability explained and the factor that contributed most positively to this axis was the distance to sea, while the variables that contributed most negatively were bacterioplankton abundances and Chl-a concentrations. Also the morphometry of lakes (Area *versus* depth ratio: Za) was associated with the negative part of this axis. The second axis explained 33.75% of variability observed. In this component, the catchment area appeared to explain differences observed in ionic composition. Thus, the basin area (Catch) was correlated positively with Mg^{+2}, Cl^{-}, and conductivity, and clearly discriminated Lake Maderos from the rest. By excluding lakes Refugio and Maderos from the PCA-2 it was possible to observe more finely the differences among the upland lakes. In this case, the first and second axes accounted for 42.2 and 28.1% of

the variability observed, respectively. In contrast to PCA-1, here the first factor was more associated with the trophic status. The variables Chl-a, lutein and bacterial numbers were more positively correlated with this axis, whereas the negative side was greatly explained by the N/P molar ratio. Concerning to the second component, strongly correlated with this axis were distance to sea and alkalinity (positive correlation), and nanoflagellates abundances (negative correlation).

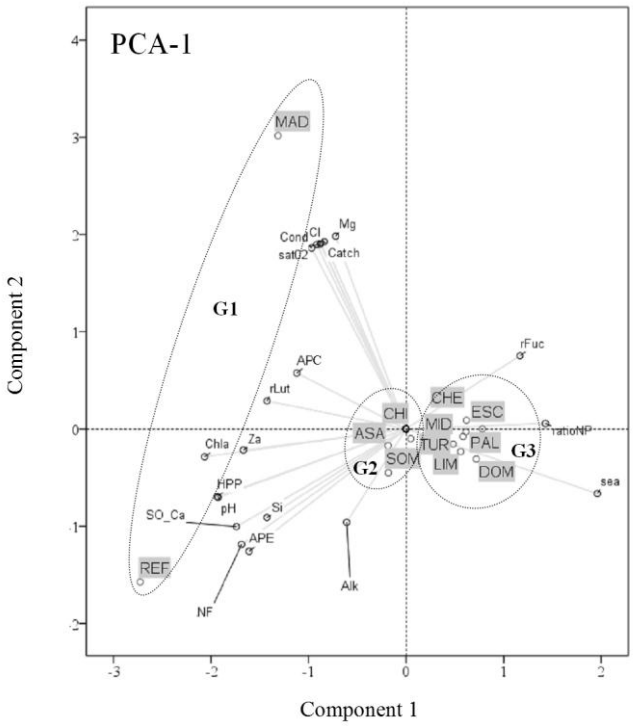

Figure 3.20. *Principal component analysis (PCA) of the physical-chemical and biological characteristics of all lakes. The lakes in PCA-1 are: Lake Aså (ASA); Lake Chester Cone (CHE); Lake Chica (CHI); Lake Domo (DOM); Lake Escondido (ESC); Lake Limnopolar (LIM); Lake Maderos (MAD); Midge Lake (MIL); Lake Las Palmas (PAL); Lake Refugio (REF); Lake Somero (SOM); Lake Turbio (TUR). The lakes in PCA-2 includes the same lakes except Maderos and Refugio (see text for details). The labels of variables are: sat02 (oxygen saturation), Alk (alkalinity), APE (autotrophic picoeukaryotes), APC (autotrophic picoplankton), Catch (catchment area), Chla (chlorophyll-a), Cl (chloride), Cond (conductivity), Sea (distance to sea), rFuc (fucoxanthin relative to Chl-a), HPP (Heterotrophic picoplankton), rLut (lutein relative to Chl-a), Mg (magnesium), ratioNP (N/P molar ratio), Na (sodium), NF (nanoflagellates), pH, Za (Area/depth ratio), Si (silicates), SO_Ca (sulfate/calcium molar ratio). Lakes from G1 and G2 envelopes lack mosses and some of them show cyanobacterial mats. By contrast, lakes enveloped in G3 show well-developed or patched mosses populations in the bottom.*

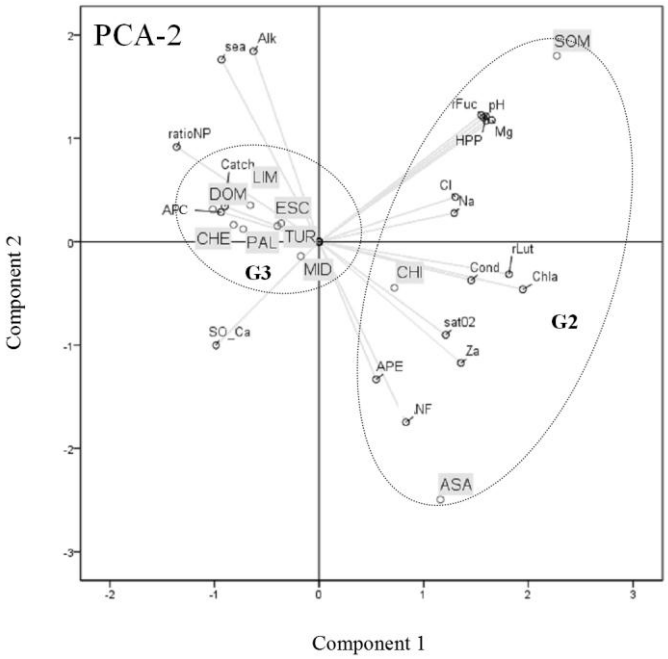

Figure 3.21. *Principal component analysis (PCA) of the physical-chemical and biological characteristics of all lakes except Maderos and Refugio (see text for details). The labels of variables are: sat02 (oxygen saturation), Alk (alkalinity), APC (autotrophic picoeukaryotes), APP (autotrophic picoplankton), Catch (catchment area), Chla (chlorophyll-a), Cl (chloride), Cond (conductivity), Sea (distance to sea), rFuc (fucoxanthin relative to Chl-a), HPP (Heterotrophic picoplankton), rLut (lutein relative to Chl-a), Mg (magnesium), ratioNP (N/P molar ratio), Na (sodium), NF (nanoflagellates), pH, Za (Area/depth ratio), Si (silicates), SO_Ca (sulfate/calcium molar ratio). Lakes from G1 and G2 envelopes lack mosses and some of them show cyanobacterial mats. By contrast, lakes enveloped in G3 show well-developed or patched mosses populations in the bottom.*

Taking in consideration the results obtained in the multivariate analysis, the relationships between some of the variables was examined. The Pearson correlation of log transformed data demonstrated a positive relationship between TP and Chl-*a* concentrations (R^2=0.83, p<0.0001; n=23), and somewhat weaker with TN (R^2=0.62, p<0.0001; n=23). By contrast, no significant correlation was observed between Chl-*a* and TN/TP atomic ratios (R^2=0.25, p=0.06; n=23). Bacterioplankton abundances were positively correlated with DOC (R^2=0.66; p=0.015; n=8), and with other trophic variables such as Chl-*a* (R^2=0.69; p<0.0001; n=24), TN (R^2=0.62; p<0.0001; n=24) and TP (R^2=0.63; p<0.0001; n=24). However, a different

relationship between algal biomass and bacteria arose depending of trophic status (Fig. 3.22). Thus, HPP numbers relative to Chl-a decreased significantly (R^2=0.75; p<0.0001; n=23) with the increase of TP concentrations. The structure of phytoplankton community was affected as well by trophic status, therefore, in the complete range of lakes studied both fucoxanthin but mainly lutein were unimodally related with Chl-*a* (Fucoxanthin: R^2=0.64, p<0.001, n=23; Lutein: R^2=0.94, p<0.001, n=23). An increase of lutein relative to fucoxanthin was observed as Chl-a concentrations increased, although not statistically significant (R^2=0.20; p=0.06, n=23). Concerning total autotrophic picoplankters (APC+APE), some interesting correlations emerged when their relative contribution to the total algal biomass was considered (Fig. 3.23). Thus, autotrophic picoplankton abundance relative to Chl-*a* concentrations were positively and negatively correlated, respectively, with TP (R^2=0.77; p<0.001; n=23) and TN/TP (R^2=0.51; p=0.04; n=23). The weakness in correlation observed in the case of N/P molar ratio was due to the deviation suffered by the more eutrophic water bodies (Refugio and the pond) which are indicated by black circles in the plots (Fig. 3.23).

Table 3.7. *Factor loadings based on Pearson correlation coefficients between variables and the dimensions of PCAs*

Parameter	Label	PCA-1		PCA-2	
		Factor 1	Factor 2	Factor 1	Factor 2
%O_2	sat02	-0.415	0.898	0.592	-0.441
Alkalinity	Alk	-0.288	-0.454	-0.306	**0.902**
Autotrophic picoeurationts	APE	-0.761	-0.594	0.267	-0.652
Autotrophic picocyanobacteria	APC	-0.529	0.272	-0.458	0.14
Catchment area	Catch	-0.396	**0.909**	-0.441	0.167
Chlorophyll-a	Chla	**-0.976**	-0.135	**0.952**	-0.225
Cl	Cl	-0.413	**0.904**	0.637	0.212
Conductivity	Cond	-0.458	**0.879**	0.711	-0.182
distance to sea	sea	**0.926**	-0.314	-0.456	**0.862**
Fucoxanthin	rFuc	0.551	0.355	0.757	0.6
Heterotrophic picoplankton	HPP	**-0.915**	-0.329	**0.779**	0.594
Lutein	rLut	-0.674	0.136	**0.887**	-0.154
Mg	Mg	-0.341	**0.936**	0.806	0.576
N/P molar ratio	ratioNP	0.674	0.026	**-0.666**	0.448
Na	Na	-0.433	**0.895**	0.631	0.136
Nanoflagellates	NF	-0.797	-0.561	0.406	**-0.854**
pH	pH	-0.910	-0.333	0.769	0.589
Relative depth	Za	**-0.788**	-0.103	0.663	-0.574
Si	Si	-0.674	-0.430	0.781	0.573
SO_4/Ca molar ratio	SO_Ca	-0.822	-0.474	-0.482	-0.49

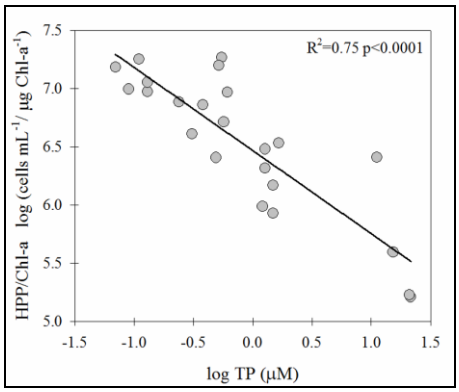

Figure 3.22. *Relationship between bacterial abundances (HPP) and algal biomass (Chl-a), expressed as the logarithm of quotient, depending of the trophic status, considered as the logarithm of total phosphorus concentrations.*

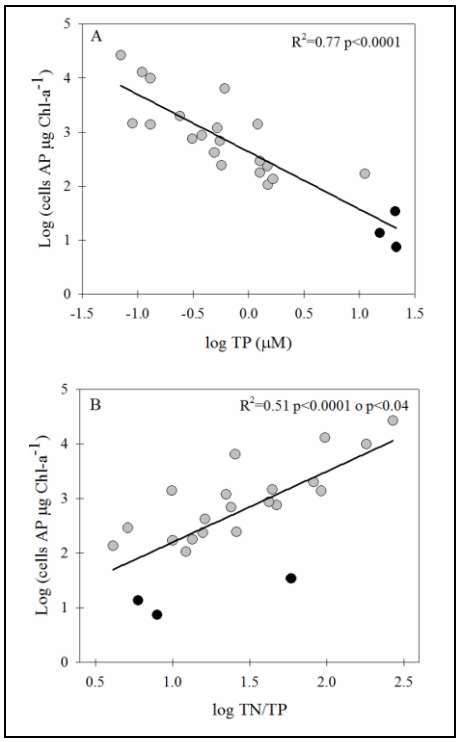

Figure 3.23. *Relative role of picoplankters in the phytoplankton community depending of the trophic status, expressed as the abundance of the autotrophic picoplankton relative to the Chl-a concentrations. A) Logarithm of the abundances of picoplankters per Chl-a amounts as function of the logarithm of total phosphorus concentrations. B) Logarithm of the abundances of picoplankters per Chl-a amounts as function of the logarithm of total nitrogen/total phosphorus molar ratio.*

3.4. Discussion

Here we have characterized a diverse set of water bodies from Byers Peninsula, which comprise a broad range of morphological, chemical and trophic conditions. With regards to the thermal regime of lakes, it is between cold and temperate thereimictic, which is similar to that reported for other sites from the maritime Antarctic region including Deception Island (DRAGO 1989), South Orkney Islands (HEYWOOD 1968) and King George Island (DRAGO 1980). This is characterized by a circulation only during summer season. Lakes in this case show a seasonal cycle similar to dimictic, although without summer stratification.

The outlets of lakes in Byers Peninsula are controlled by several factors during summer period, such as snowmelt, rainfall events, surface water evaporation and permafrost melting. As observed in some particular cases, an important decrease of water level may occur in very short periods due to the ice-dam break-up. These dams are formed by the freezing of the lake outlet together with a huge accumulation of snow. It implies a certain inter-annual variability since the extent of the outlet blocking depends on the amount of snowfall accumulated during the preceding winter. Hence, it would result of interest to go deeper into the phenomenology of this process given that they are expected to affect profoundly the physical structure of lakes.

The biomass of pelagic primary producers in these lakes is very low in general; we can then to expect oxygen profiles largely determined by temperature and salinity. Nevertheless, there are additional considerations that can be made. The vertical distribution of oxygen was essentially orthograde in all the lakes profiled, which agree with their oligotrophic conditions. This is due to reduced oxygen consumption, summed to some oxygen evolution produced by the benthic flora. Also, the basin shape and the deepness of some lakes likely contribute to create these profiles. However, in some cases oxygen decreases with depth, such as observed in the early sampling performed in Lake Turbio, thus suggesting some oxygen depletion during winter. Probably, larger lakes (i.e. Midge Lake) keep sufficient oxygen concentrations during winter, by contrast, in those lakes shallower that furthermore have higher organic loads, certain oxygen depletion may occur below the ice cap during winter. Moreover, other factors such as the heat content of bottom sediments have been found to regulate the oxygen concentrations in shallow ice-covered lakes (TERZHEVIK ET AL. 2009).

The highly oxygenated conditions occasionally observed exceed the values expected from temperature, which can result from a number of causes. This could be

partially explained by the low degree of salinity of lakes. Otherwise, also the ice-cover of lakes might cause gases oversaturation (CRAIG ET AL. 1992). This is because the ice cap prevents much of the exchange of oxygen with the atmosphere. However, lakes were nearly partial or complete ice free during survey. Another explanation would be the existence of a significant photosynthetic activity of the benthic mosses, which might produce enough oxygen to cause supersaturation. This benthic primary production is suggested for instance in the profile made in Midge Lake at December. There, it was observed a slight increase of pH with depth, which usually is the consequence of the take-up of CO_2. There are, however, some exceptions, as in the case of Lake Refugio, which shows oxygen concentrations below saturation (around 75%). As noted previously, it might respond to the biological demand originating from the inputs of organic matter owing to marine animals. On the other hand, slight subsurface peaks of oxygen were detected in some profiles, likewise resulting from the photosynthetic activity exerted by phytoplankton.

Based in our findings, waters from Byers can be catalogued as the sodium type. With regards to anions, most of them are not of any dominant type, nevertheless some lakes such as Maderos, Escondido and Aså show a significant dominance of chloride. The slightly acidic pH and the low alkalinities are also a regular feature. This implies a lower buffering capability for neutralize the inputs from the bedrock dissolution, or even the rainfall, which is moderately acidic due to the presence of dissolved CO_2. The bedrock in Byres Peninsula is mainly formed of igneous material and catchments of the lakes are covered by acid lithosols, which may explain the low neutralising capacity of water. However, the soil development may also account for this acid character. In this sense, it has been observed a tendency of lakes acidification in recently deglaciated areas as a result of the progressive weathering of soluble minerals from immature soils (ENGSTROM ET AL. 2000).

The water acidification of nutrient-poor system has been also explained by a depletion of basic cations (e.g. Ca^{2+}) greater than its replacement (BIRKS ET AL. 2000), which can be accentuated because its sequestration by the terrestrial biomass. The Ca/Cl equivalent ratios observed in lakes from Byers, with the exception of Lake Refugio, are all considerably higher than the seawater ratio (0.038), thus suggesting that the catchment leaching prevails over the sea influence. However, it does not imply necessarily an elevated rate of calcium weathering. Otherwise, an eventual slight acidification of lakes might occur by the washing of humic substances. This idea is in part sustained by the occurrence of lichens and microbial

mats in the catchment, which produce secondary metabolites (e.g. usnic acid, fulvic acids). Contrastingly, higher pH occur in coastal lagoons as in Lakes Refugio and Limícolas (7.7 and 7.8 respectively), likely as a consequence of higher photosynthetic rates, and accentuated by the relatively low buffering capacity already mentioned.

In some lakes being sampled several times, an increase of conductivity during the summer season was observed. This is reflected in the concentration of some ions, yet, they were very conservative and varied narrowly. A progressive increase in salinity concentrations as summer advances seems to be a common trend in polar lakes (BORGHINI ET AL. 2008 and articles cited therein), which responds to the enhancement of drainage occurring during melting. Furthermore, this agrees with a predominant role of the rock/soils weathering. In this sense, the weathering of catchment material seems to be, in relative terms, more important in the central part of the plateau, given that lakes located there show the higher relative proportions of Ca^{2+}. As noted by LYONS ET AL. (in preparation) an excess of Ca relative to Na in lakes from Byers can be interpreted to be derived from mineral weathering (i.e., $CaCO_3$ dissolution). These authors found the higher content of Ca in streams originating from the inner part of the Peninsula, which was explained by the lithology. By contrast, in our case, both the coastal lagoons and those lakes from the plateau situated easternmost share the lower proportions of Ca^{2+} relative to Na^{+}. These differences can be affected in part by the sequential predominance (from west to east) of less developed soils as they are near to the glacier front. However, it could be also a consequence of a differential sea influence.

The relative enrichment of Na^{+} indicated in the Gibbs diagram suggests, in any case, that waters are not in equilibrium with the surrounding catchment. In particular, it is reflected in the higher Na^{+} content relative to Ca^{2+}, which is higher than the expected by the rock weathering. Similarly to our findings, there are data sets inconsistent with the Gibbs boomerang model which generates outliers (WU AND GIBSON 1996 and articles cited therein), thus proving that there are other sources that may explain the chemical composition of waters. It is certain that the Gibbs model applies well to the major waters in the world on a regional scale, however, the chemistry of small lakes might be more affected by local factors. In our case, both the rock weathering and tropospheric precipitation alone are unable to explain the observed Na^{+} enrichment. The overall chemistry of lakes from Byers is likely affected by the sea spray, which may explain the chloride and sodium enrichment. Indeed, the Na/Cl ratios in lakes rang 0.87-1.63, being in several cases near to sea ratio (0.86). This idea match furthermore with the fact that lakes more

deviated from the Gibbs envelope are indeed those located in the coast (Maderos and Refugio). By contrast, the lakes showing similar weight ratios [$Na^+/(Na^+ + Ca^{2+})$] but very low conductivities are those sited in the eastern part of the Peninsula, beyond the Chester Cone (Las Palmas, Escondido, Domo), which probably are more sheltered from the sea spray.

Both nutrients and Chl-*a* concentrations underlie the productivity gradient found from inland to littoral lakes, which is defined in the first component of the PCA-1. Two main limnological regions are observed: a central plateau showing many medium-depth and shallow lakes with small watersheds and ultra-oligotrophic, and the coastal area that embraces the north, south and western beaches, where water bodies commonly shows high nutrient concentrations. In some trends, our findings match with a previous study performed by JONES ET AL. (1993) in the region, which also included lakes from Signy Island. The water bodies from Byers in that case also segregated in function to their trophic status and marine influence. However, it seems that lakes are differently discriminated in our case, in part because the study of Jones and co-workers focused on benthic diatoms. Thus, we distinguish at least three different lake groups. The first group includes the coastal lagoons, which are positively associated to trophic indicators. These water bodies experience heavy nutrient inputs because of the activity of fauna in their vicinities (mammals and birds), principally southern elephant seals (*Mirounga leonina*). They have, in addition, a higher exposure to sea-sprays, which might provide an additional supplement of nutrients. The marine intrusion might, in some cases (i.e., Maderos), intensify these inputs. Other group is the composed by mid-depth and shallow (0.5~3 m) lakes located more inland (i.e., Somero, Aså and Chica), in which a strong sediment-water interaction occurs. Here, the wave-induced re-suspension of sediments favoured by the shallowness of lakes likely enhances the input of nutrients. This nutrient turnover also occurs in the coastal lagoons, however, it should be more important in the former, particularly in the shallowest Lake Somero, where external sources are of less importance compared to internal loads. The sediment removal occurring in these lakes is reflected, for instance, in the higher amounts of pheopigments relative to Chl-a, reflecting resuspension of already settled algae with pigments showing a higher degradation. The burrowing activity of benthic invertebrates, principally *Branchinecta gaini*, which are quite abundant in shallow lakes from the plateau, is also likely contributing to this sediment recruitment. Finally, the third group includes deepest lakes (5 to 9 m) located furthest from the coast, which receive limited inputs of nutrients thus rendering them oligotrophic.

The mosses coverage of lakes' bottom is not included in multivariate analysis because we failed to do an appropriate quantitative survey. However, visual observations indicate that it could be an important attribute also rendering variability among lakes. In this sense, the first and second groups of lakes discriminated in the PCA (G1 and G2 envelopes in figure 3.20 respectively) lack benthic coverage of mosses, though, some of them show cyanobacterial dominated mats in their shores. By contrast, most of the lakes of the third group (G3 in figure 3.20) show well-developed or patched mosses populations in the bottom. This matches with the idea that both deepness and sediment stability are desirable conditions for the setup of these benthic communities. On the other hand, it is noteworthy how in lakes lacking mosses the microbial mats show also a restricted distribution, forming a ring along the lakesides (i.e., Lake Somero). This differs with the observed in lakes from other regions of Antarctica where mats dominate in the benthos because the high scarcity of metazoan (QUESADA ET AL. 2008). Yet, the impact of the reduced light conditions, especially during the ice cover period, should be also considered. On this sense, it seems that some morphological adaptations of mosses allow them to be more efficient in capturing light (WALTON AND DOAKE 1987). At light intensities below the compensation point, the respiration rates in mosses could be in relative terms lower compared to that of microbial mats. Otherwise, the slow accretion of mats likely requires low disruption for setting up a significant coverage. Therefore, the development of these mats in the shallower lakes is impeded by the instability that wind induces. Although they dominate in other habitats from Byers (see chapter 7), it seems that there are several stressors preventing that mats colonize the lakes' bottom.

Differences in lake's chemistry can be explained by differences in the mineralogy of soils. Earlier stratigraphical studies performed in Byers reveal a variable sequence along the Peninsula (SMELLIE ET AL. 1980). Basically, this consists of marine and terrestrial sedimentary deposits in the west and east areas respectively, with an intermediary region between them. Marine sandstones and mudstones mainly compose the western part. Towards the east, it derives fast in a mixed area with volcaniclastic sandstones and conglomerates. Beyond, from the Chester Cone inclusion until the eastern part of the Peninsula, the bedrock is mainly composed of pyroclastic rocks. Considering this stratigraphy, trends of silica concentrations in waters likely respond to irregularities in the allocation of tephra deposits. These variations in the catchment geochemistry has been already reported by JONES ET AL. (1993), which also indicates the occurrence of higher amounts of silica in lakes from Livingston Island compared to those from Signy Island. A revision of the SMELLIE's work carried out by HATHWAY AND LOMAS (1998)

introduces considerations of the stratigraphy of Byers allowing to better understanding these dissimilarities. Thus, lakes with lower silica concentrations (Escondido, Las Palmas and Domo) are settled on the Cerro Negro Formation, which is composed by non-marine volcaniclastic rocks. According to HATHWAY AND LOMAS, this is a volcanic stratum with a largely silicic lower division, however, its upper part has a basaltic origin composed mainly by tuffaceous breccias (tephra), which shows low silica content. Attending to the TAS classification (LE MAITRE 2005), these types of deposits are even low in silica (only around 40% SiO_2) than other igneous rocks.

The chemical weathering in Antarctic catchments can be similar in magnitude to that accounted in temperate areas (NEZAT ET AL. 2001). In studies performed by LYONS ET AL. (1998) in Dry Valleys, authors found that the weathering of stream deposits jointly with the atmospheric deposition provided most nutrients and solutes to the lakes, so that ion concentrations were in general positively related with the stream length. However, in our case is not evident a simple relationship between the concentration of major nutrients and the catchment size. The inlets in Byers are maybe short in general to produce a significant nutrients leaching. Furthermore, it must be noted that LYONS and co-workers studied a region with very low precipitations, which differs greatly with Byers. Differently to Dry Valleys, likely the role of atmospheric inputs in Byers is equivalent or even higher to the catchment contribution.

The gradient of productivity observed in lakes seems to demonstrate the prevalence of a resource limitation for pelagic production, both for heterotrohic and autotrophic populations. This relies in the idea that both covariate depending of the ratio between organic carbon and major nutrients (HULOT ET AL. 2001). It is noteworthy that bacterial numbers that we observe are moderately high regardless of lakes' oligotrophy. In lakes with lower productivity, in which heterotrophic metabolism exceed the pelagic primary production, the benthic communities growing in the catchment can supply bacteria (CAMACHO 2006a). In this sense, the DOC concentrations in streams from the McMurdo Dry Valleys has been observed to be lower (< 80 μM), but they are around 10-folds higher to those directly originating from the ice melting (LYONS ET AL. 2007). This has led to some authors to presume that a great part of this DOC derives from the primary production taking place in the catchments (VINCENT AND LAYBOURN-PARRY 2008). If so, this implies that bacteria are mainly subsidized by allochthonous carbon, supporting the idea that low-productive systems tend to be net heterotrophic (TAKACS AND PRISCU 1998, JANSSON ET AL. 2000). We are in agreement with this idea, but we cannot rule out

the existence of authoctonous sources as those originated from the submerged populations of aquatic mosses (and the periphyton attached to them), particularly in those lakes in which these populations dominate the benthos.

Other idea arising from our findings is that phytoplankton and bacteria may develop either competitive or commensalistic interactions depending of the nutrient status. This relies in the fact that bacterial production in oligotrophic ecosystems can be limited by inorganic nutrients apart from organic carbon (FISHER ET AL. 2000), which could be the case of some lakes in Byers Peninsula. Bacterial numbers and DOC roughly covary in our case (Fig. 3.24), however, it is possible also that N and/or P availability regulate the DOC consumption. This could be the case, for example, of lakes Limnopolar and Domo, in which a phosphorus deficiency is evident. On the contrary, lower N/P ratios and high DOC concentrations occur concurrently with the higher bacterial numbers. The idea that can be extracted is that even having enough carbon supply for bacteria, its use appears to be limited by the phosphorus availability, which indeed agrees with the observed in other Antarctic lakes (SAWSTROM ET AL. 2007b).

The relative dominance of algal groups in the lakes can be deduced from trends of taxa-specific carotenoids. Although the relationship is somewhat weak, the increase of trophic status seems favouring a progressive dominance of chlorophytes over other algal groups (chrysophytes and/or diatoms). However, most of these diatoms have a benthic origin and therefore their abundances are not always a direct response to trophic status, but are mainly related with external inputs via runoff. With regard to chrysophytes, they have been regularly associated with oligotrophic waters both in mountain lakes (CATALAN ET AL. 2006) and in lakes from high latitudes (IZAGUIRRE ET AL. 2003; FORSSTRÖM ET AL. 2005). On the contrary, it has been noted how in nutrient-impacted sites some chlorophytes groups (flagellates and/or coccoid unicells) can become important (SIGEE 2005).

The nutrient status of lakes appears to affect the relative abundance of autotrophic picoplankton. As noted in the Spearman correlations, both TP concentration and TN/TP ratios are in our case good predictors of the relative role of autotrophic picoplankters in the phytoplankton assemblage. Thus, a clear trend of reduction in their abundances relative to Chl-a is observed as trophic status increase, in such way that relationships with TP and TN/TP are negative and positive, respectively. The occurrence of low TN/TP ratios associated with increasing trophic status is a regular trend in inland waters (WEHR 2008), even in Antarctica (BORGHINI ET AL. 2008). Apparently, this also occurs in Byers and, tentatively, determines the prevalence of a nitrogen limitation when phosphorus is in excess,

such in the case of coastal lakes affected by marine animals. This could be true at TP levels up to 2 µM, seeing that the linear relation between TP concentrations and TN/TP apparently saturates when the former are higher. The increase of the relative importance of photosynthetic picoplankters at low nutrient levels has been previously reported, in both field (VÖRÖS 1991; AGAWIN ET AL. 2000; CAMACHO 2006b) and experimental studies (WEHR AND CAMPBELL 1994; LAGUS ET AL. 2004). This has been partly explained by the competitive advantage that the smaller autotrophic picoplankters have over larger cells for nutrient assimilation (BELL AND KALFF 2001) because they have a larger cell surface/volume ratio. Still, its contribution to the total algal biomass was generally low in our case.

Special attention should be paid to the peaks of autotrophic picoeukaryotes observed occasionally in the surface of deepest lakes such as Midge Lake and Chester Cone. This may respond to their higher photosynthetic and growth efficiencies at low light levels compared to larger phytoplankton (RAVEN 1998). Forms similar to picoprasinophytes composed these populations. These pico-sized shade-adapted organisms are known to progress in polar seas (LOVEJOY ET AL. 2007) and also in Antarctic lakes (BELL AND LAYBOURN-PARRY 1999b, 2003). Differently to other algal groups, apparently these picoeukaryotes persist just beneath the ice cap during late winter despite of the reduced light availability, just when radiation transmission increases due to the progressive reduction of the ice cover. This would explain that, although to be probably common in other lakes from Byers, we only observed them in lakes where the ice thaw occurred later. In spite of these sub-superficial peaks, in no case a development of significant deep rich layers of small autotrophs were observed in our study during the ice-free period. Following the idea of CAMACHO ET AL. (2003), the low vertical stability in Byers' lakes from Byers, mainly after the ice melting, combined with their relatively shallowness likely represents unfavourable conditions for the formation of these high productive sub-superficial layers.

Concerning zooplankton we report here remarkable low values of diversity. This contrasts with the observed in other habitats from the same site (PETZ ET AL. 2005, 2007). Thus, the greater diversity of ciliates in Byers for instance (up to 120 species described) corresponds with benthic environments, both in running and stagnant waters. In our opinion, this is due to the lesser uniformity of benthic environments compared to the pelagic ones, thus providing a suite of different habitats that facilitates a higher diversification. Among the metazoan, they are species commonly reported in the maritime Antarctica region such as *Boeckella poppei* (BAYLY ET AL. 2003; IZAGUIRRE ET AL. 2003) and *Branchinecta gaini* (PECK

2004). The thriving of the anostraca *B. gaini* in Antarctic lakes requires the presence of well-developed benthic communities or, at least, the occurrence of organic rich sediments due to pelagic inputs (BJÖRCK ET AL. 1996). In Byers, we verify the regular occurrence of *B. gaini* in lakes, both in deeper lakes containing benthic mosses populations and in shallowers, where benthic mats are occupying solely the margins but in which sediments are supposed to be rich in particulate carbon as a consequence of a higher productivity. Anyhow, the sampling methodology used in the deeper lakes (hydrographic bottles) does not allow us to obtain benthic samples accurately. It is for this reason that it is not possible to establish precise trends of anostracean distribution. With all, it seems that the primary production in lakes drives the distribution of *B. gaini* as is suggested by its higher abundance in shallower lagoons with a higher nutrient content, in which organic detritus in sediments originated from plankton sedimentation is supposed to be more abundant.

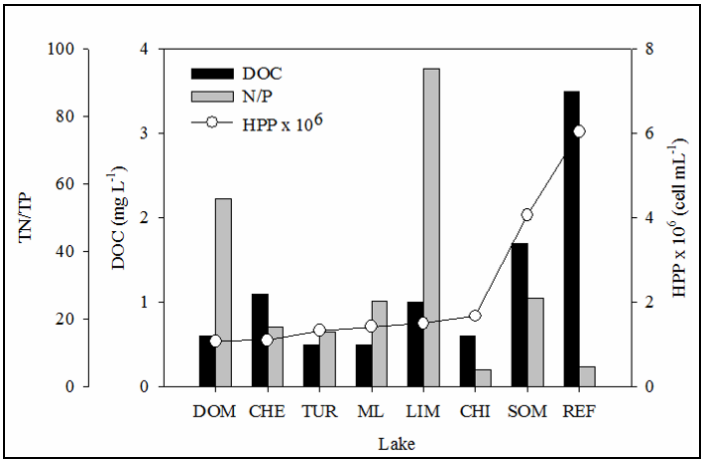

Figure 3.24. *Concentration of dissolved organic carbon (DOC), N/P molar ratios and bacterial densities (HPP) measured in different lakes from Byers Peninsula during ice-free summer period. Lake Chester Cone (CHE); Lake Chica (CHI); Lake Domo (DOM); Lake Limnopolar (LIM); Midge Lake (MIL); Lake Refugio (REF); Lake Somero (SOM); and Lake Turbio (TUR). The lakes are ordered in ascending bacterial abundances.*

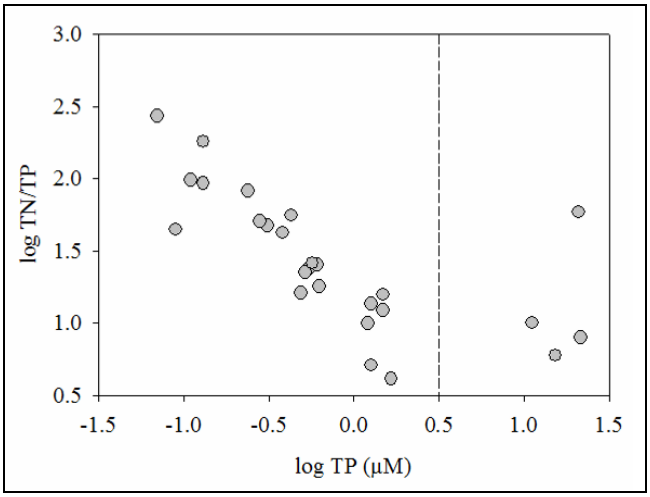

Figure 3.25. *Relationship between log TN/TP molar ratio and log TP concentrations (µM) in lakes of Byers Peninsula.*

We have conducted the metazoan sampling with net hauls, therefore, the densities of copepods reported here are approximations that do not allow to extract comprehensive conclusions. With all, it results evident that *B.poppei* composes most of the pelagic community of metazooplankton in lakes. An overwhelming dominance of *B. poppei* has also been observed in lakes of Hope Bay in the Antarctic Peninsula (IZAGUIRRE ET AL. 2003). Also, in lakes from Amery Oasis (East Antarctica), this copepod was the only crustacean found in the water column (BAYLY ET AL. 2003). Certainly, rotifers are also present in Byers, however, they seems to be scarce, at least during the studied period.

We observe timing in the life-cycle of *B.poppei* among lakes. In this sene, a nauplius phase occurs at early summer while adults dominate at the final of period (Fig. 3.16), which agrees with the observed in other sites from the maritime region (UNREIN AND VINOCUR 1999 and articles cited therein). These authors establish a univoltine life cycle for this specie, that is, a single reproductive event during year. Nevertheless, the occurrence of several reproductive events during the same year has been also occasionally demonstrated (IZAGUIRRE ET AL. 2003). If univoltine life cycle occurs, the sexual activity in the cohort is higher just before the commencement of summer, when lakes are still covered by ice, and is followed by a peak of adults through the late summer. Apparently, they spend the rest of the year in a dormancy state, waiting for the onset of lake's productivity. The synchrony that we observe points to an endogenous control of development, which in other

copepods (*Calanus finmarchicus*) has been suggested to be controlled by a programmed response occurring during a particular moult (HIND ET AL. 2000).

In summary, we demonstrate the occurrence of a relatively large productivity gradient that extends from the lakes located upland, which are characterized by small watersheds and ultra-oligotrophic conditions, to the coastal water bodies located close to the sea, which show higher salt and nutrient concentrations. The lakes' morphometry also accounts for differences observed, in such a way that the shallowness favours the internal inputs of nutrients, which is drawn by the positive covariance observed in the PCA's analyses between the algal biomass and the ratio between area and depth (Za) of lakes. In lakes located in coastal areas the internal loading is overpassed by the occurrence of external sources. We have shown some aspects of the biotic structure, mainly related with microbial populations, depending of this increasing trophic status. Thus, it appears that nutritional status of lakes clearly play a significant role in structuring the pelagic communities despite of climatic constrains, however, the existence in some cases of a top-down control must not be discarded as we will try to demonstrate in following chapters.

4. Physical and chemical features of Lake Limnopolar in three meteorologically contrasting summers

4.1. Introduction

Dynamic thermal structure is a regular feature of lakes. The physical processes involved are primarily a function of the atmospheric variables, however, in-lake characteristics may also account for the observed thermal regimes. Vertical mixing, for instance, which depends on the lake morphometry, is an important factor that modifies the vertical distribution of heat. There are studies devoted to explore the heat exchange occurring at the air-water interface in Antarctic lakes (ELLIS ET AL. 1991, REID AND CROUT 2008), but they are essentially limited to the continental region. Therefore, little information is available for lakes located in the maritime region, where different findings are expected because the both regions differ in their climatological characteristics. Thus, in contrast to the continental region, where the homeostasis caused by permanent ice cover results in unvarying physical and chemical gradients in lakes (SPIGEL AND PRISCU 1998; ROBERTS ET AL. 2000), the climate in the maritime region allows for ice melting in the summer. The acquisition of new field data in this region will hence improve our knowledge of the physical dynamics of these lakes.

The heat budget of a lake is the balance of the heat transferred between their waters and the atmosphere. It accounts for energy gains and losses, which originate from different sources. The main sources of energy flux are solar and long-wave radiation, latent and sensible heat, and the transfer of heat produced during rainfall. Solar radiation is comprised of a wavelength spectrum containing light from ultraviolet (200-280 nm) to infrared (longer than 760 nm), which therefore includes photosynthetic active radiation (PAR; 400-700 nm). Long-wave radiation is emitted by the Earth's surface and involves a wavelength spectrum from 5000 nm to about 100,000 nm. Absorption of solar radiation by the lake (also called short-wave radiation) implies a gain of heat, whereas long-wave radiation releases heat from the lake's surface to the atmosphere.

Both latent and sensible heat is turbulent energy fluxes which, in the end, are derived from solar radiation. Latent heat in a lake involves the flux of energy between the air and surface water, produced from a change in the state of matter. As it is associated with freezing and melting process, latent heat may be important during certain periods in lakes at high latitudes. On the other hand, sensible heat is a flux produced by a conduction or convection event, in which an excess of heat is transferred into the atmosphere. Because both fluxes are a function of the turbulence produced by the wind, they can be estimated using a bulk aerodynamic formula (MOMII AND ITO 2008). In addition, atmospheric precipitation may also induce

sensible heat flux. This flux is great when large differences in temperature occur between the rain and the soil or water surface, though notably, it is commonly neglected in the heat budget analysis. Other heat fluxes are those associated with the inflow and outflow of water mass in the system. Finally, the balance of all of these energy flux events yields the heat budget in the lake.

Heat flux is largely suppressed when the lake surface is frozen. This is the case, for instance, of the solar radiation penetrating into the lake or the shear produced by wind on the lake surface. Sensible heat loss is notably prevented, as both ice and snow are poor heat conductors. In addition, the translation of energy into the lakes takes time, which entails a delay between atmospheric forces and their effect in the lake. This is because the heat capacity of water allows even small lakes to accumulate heat. In particular, snow enhances the reflection of solar radiation due to its elevated albedo, which not only discourages the lake ice from thawing but also prevents freezing during ice formation. Otherwise, the ice formation involves a release of latent heat (i.e., exothermic process). Due this strong isolation, other physical processes prevail in the lake. Such processes include, for example, baroclinic currents that may result in nutrient and seston redistribution in the water column (KENNEY 1996).

Causes that explain the characteristics of ice cover on lakes during summer periods extend to the severity of the previous winters. Temperature at the time of ice formation therefore may explain the lake's optical characteristics, which, finally, affect the amount of light that reaches the lake surface. It is known that faster freezing produces a higher abundance of bubbles in the ice (CARTE 1961); though, a more homogeneous nucleation and therefore a higher transparency occurs when ice is formed slowly. The amount of winter snow accumulation will thus determine the condition of the ice in the lake. However, factors other than atmosphere that might account for the heat budget in the lake are morphometric characteristics. Most of the lakes in Byers Peninsula, including Lake Limnopolar, show a particular fluvioglacial landform characterized by kettle morphology, which may be a legacy of their geological origin (BENNET AND GLASSER 1996). The chronological scheme for the formation of this kind of lake is depicted in figure 4.1. During glacier retreat, certain depressions became filled with blocks of ice from the front of glacier. Afterwards, this ice melted and a depression containing water was formed. Depressions able to retain water became kettle lakes or wetlands. This could be the geological origin of Lake Limnopolar, however, in some cases this process is not easy to define because certain other mechanisms are easily confused with kettle formation (LÖFFLER 2004).

In any event, this morphology has important consequences for the thermal and mixing patterns in the lake, such that it may provide a shelter from the entire mix.

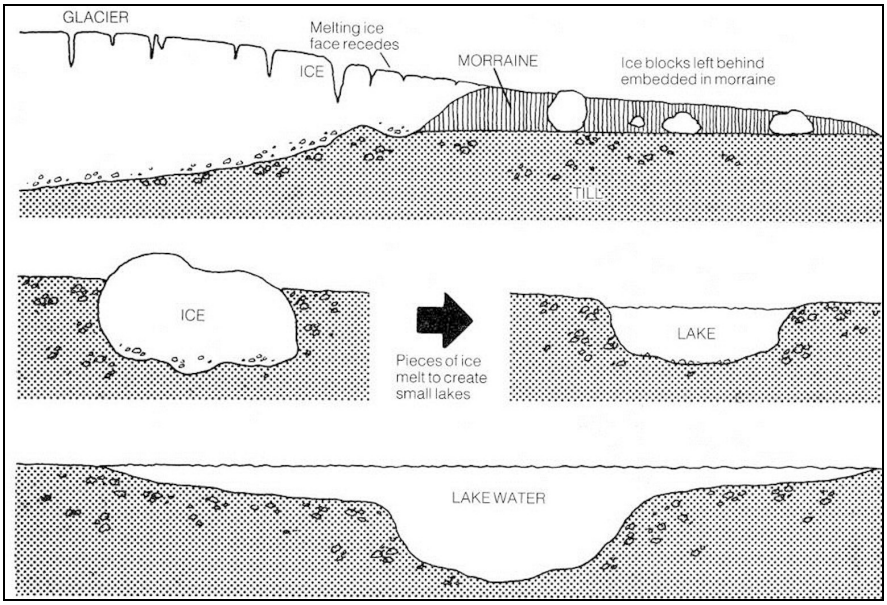

Figure 4.1. *Scheme showing the sequential formation of a kettle lake. It is a type of lake formed during the recoil of a glacier. After the glacier retreat, some ice blocks keep retained in the moraines. After melting, they left a depression in which a lake will form if the level of the groundwater is above the basin level. The deeper area within the lake is termed the kettle hole. This morphology resembles that of many lakes of Byers Peninsula, including Lake Limnpolar. Extracted from* BURGUIS AND MORRIS *(1987).*

Water movements transport the chemicals between the different depths in a water column. The study of turbulent water movements may be quite complex; however, the coefficient of eddy diffusion, as derived from the Fick's Law, can be useful to approximate water motion in routine limnological studies. This is a parameter inversely related to water column stability and depicts the progress of turbulent energy within the lake (JASSBY AND POWELL 1975). Eddy diffusion can be directly obtained by following temporal and spatial changes in the distribution of a conservative chemical tracer (VON ROHDENA ET AL. 2009), but can be also defined based on the heat flux gradient method (ELLIS ET AL. 1991; RAVENS ET AL. 2000). If the latter method is used it renders a thermal diffusivity coefficient (K_Z). In practice, the coefficient K_Z can be considered as the velocity at which heat flows within the water column. To obtain this parameter, one must analyze temperature changes in

profiles subject to transient thermal conditions. The diffusive flux of nutrients in the water column can be tentatively inferred from the *Kz* calculation, as diffusion is driven by gradients and *Kz* is analogous to molecular diffusion.

Inter-annual climate variations in the Antarctic Peninsula and the maritime region have been found to be higher compared with the continental region, which seems to be linked with sea ice content (KING 1994). These variations are supposed to affect the energy fluxes previously described. The lakes undergoing seasonal thawing and freezing, such those of the maritime region, should thus display important temporal changes in their temperature-density profile. For example, certain lakes from Decepcion Island (62°58′37″S 60°39′00″W), located very close to Byers Peninsula, have been classified as pleomictics (DRAGO 1989), which denotes that thermal overturn occurs continuously during the short summer period. Anticipation or delay of ice thaw is expected to modify the thermal regime of these lakes. We hypothesize that terrestrial and limnetic ecosystems from the Antarctic maritime region are very sensitive to small changes in air temperatures because of the milder summer temperatures found in the region, which are very close to the freezing point. In this way, small variations in air temperature can promote important changes in the ice cover and, thus, in the physical lake dynamics during the spring-summer period, when melting occurs.

To explore these ideas, we analyzed the temporal variation of temperature structure and water column stability observed over three consecutive summers in Lake Limnopolar, during which the climate conditions and, therefore, the ice cover dynamics differed significantly. The concentration of major nutrients was also measured during these periods. Due to this climate variability, summers of 2001-02 and 2002-03 were mainly ice-free periods, whereas most of the summer 2003-04 survey was conducted with the lake still covered by ice. Winter snow accumulation also differed between these years, which could also play a role in the summer melting dynamics both of the lake ice-cap and the snow cover within the catchment. The latter greatly influences the lake's hydrology, and consequently, attention must be paid to winter meteorological features to understand the summer lake's dynamics.

4.2. Methodology

4.2.1. Study site

The present study was performed in Lake Limnopolar (see chapter 3 for further information). It is a small lake with a maximum depth of 5 m located 90 m a.s.l. on the South-Western part of the central plateau of the Peninsula (Fig. 4.2). The lake has a bared drainage watershed of 0.581 km^2. Most of the catchment run-off flows into the lake through a little stream, although undefined inflows can also be important during the snow melting. There is another small drainage lake within Lake Limnopolar watershed, Lake Somero, which is located 200 m upstream. Its maximum depth is 0.7 m and its outlet flows and mixes up with the inlet water of Lake Limnopolar (Fig. 4.2). The bottom of Lake Limnopolar is covered by the aquatic moss *Drepanocladus longifolius* (see chapter 3 for a further description). This monospecific moss carpet occurs at depths of 2.5-5 m, covering almost all the lake bottom.

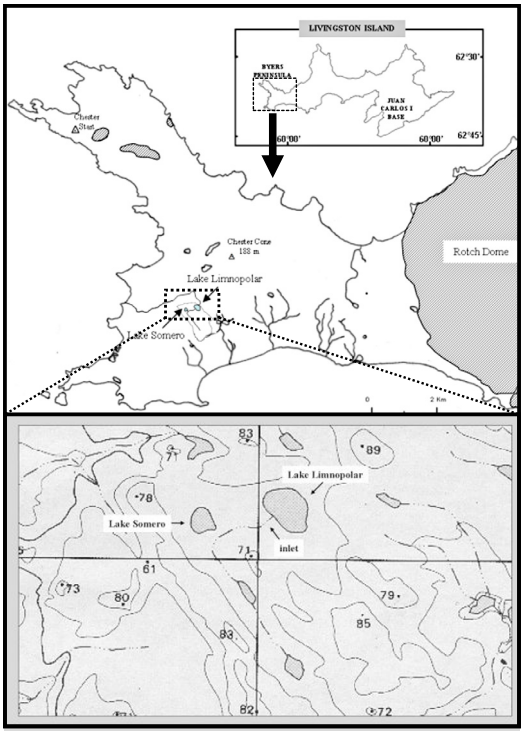

Figure 4.2. *Location of Lake Limnopolar and its watershed in Byers Peninsula (Livingston Island).*

4.2.2. Meteorological data and freeze dates estimation

The meteorological characteristics in Juan Carlos I Polar Station were measured with an automatic meteorological station equipped with a SEAC (until 2005) and a Geonica (from 2005) datalogger and temperature, humidity sensors at 1.5 m above the ground, wind speed and direction at 10 m. Data were stored every 10 min. The extended summer temperature data (November to February, both included) from this station were measured from summer 1987-1988. Meteorological data in the lake site were obtained from an automatic meteorological station (AMS) located between lakes Limnopolar and Somero, at 2 km from the coast (For technical details of this AMS see section 2.2.1).

Lake ice-off dates, defined as the first day on which the main basin of the lake was ice free, were determined by visual observations. Variations in the thickness of snow, ice and water level were also monitored during summer 2003-04. After the end of February, no human presence was possible in Byers Peninsula because of logistical constraints. Consequently, we followed the modelling ideas on CAHILL ET AL. (2005) to estimate freeze dates, defined as the first day with a covering of ice on the main basin that did not thaw for at least three days. The freezing modelling was based on assuming different water temperature fluctuations before and after ice covering, measured in our case by the standard deviation of the five subsequent mean daily temperatures. When the lake was ice covered, the deviations fell down and stabilized near to zero. Therefore, we estimated the freeze date by locating the breakpoint on the level of the 5-day standard deviation series between two thaw dates. The breakpoint corresponds to the day that the sum of squared residuals was minimum assuming different linear models at both sides of the breakpoint. Our seek of the freeze date was limited to the days with ≤0 ºC mean water temperature.

Differently to Lake Limnopolar, the close proximity of Lake Somero to the AMS made possible to install a probe to register temperatures in continuous during the three consecutive years. By contrast, to obtain a continuous temperature record in Lake Limnopolar was only possible at summer 2002, when it was observed a match between the results obtained for both lakes (see section 4.3.2.4). It is for this reason that the freeze dates in Lake Limnopolar for the following years was approximated based on the observed for Lake Somero. We assume that lake depth has an influence on ice dynamics, however, it has been found to cause variations of around only 1 week in the mean value of the ice-in dates (STEFAN AND FANG 1997).

An important increase on water temperature was observed in April 2003, indicating a possible second thaw period just before the meteorological station stopped recording data. In this case, we predicted the missing data between April 24 and May 3 by a nonparametric Lowess estimate. Then in 2003 we estimated two freeze dates, separated by a period of almost two months.

4.2.3. *In situ* measurement of physical and chemical parameters

The limnological work was carried out in Lake Limnopolar over three consecutive summers from December 2001 to February 2004. The routine sampling site was located on the point of maximal depth. Sampling for chemical analyses and profiles of temperature, conductivity, and photosynthetic active radiation (PAR) was performed as described in section 3.2.1. When the lake was covered by ice, the sampling devices were lowered through a hole drilled in the ice using a motorized ice auger. For the PAR measurements, the holes were covered with snow to avoid light entering; consequently, the obtained profiles were the diffused radiation transmitted through the ice and snow sheet. We assume that the scattering produced by ice was strong enough to offset the possible shading caused by our procedure. Using the data rendered by the light profiles, we obtained the attenuation coefficient for PAR (K_{PAR}) from the Lambert–Beer law (see section 2.1.3). When the ice disappeared, all sampling processes were carried out from a boat on the same point.

The same CTD probe used for the vertical profiles was left floating in the lake at 2 m depth with an anchored buoy. The logger was programmed to collect data at intervals of 1 hour. This same procedure was followed exactly over the three consecutive summers. During summer 2001-02, the CTD was installed from December 28th to January 11th, over summer 2002-03 it was from January 15th to the 30th, and over summer 2003-04 it was from December 30th to January 23rd. The CTD was checked several times during these continuous records for correct functioning, and, if required, then the oxygen sensor membranes were changed and the probe calibrated again.

4.2.4. Bathymetric survey

A bathymetric map was made for Lake Limnopolar during the ice-free period of summer 2002-03 using a GPS and a deep sounder. A total of 10 transects were observed to draw the contour map and highlight the main features of the lake

morphology. With this data, isopleths were drawn at intervals of one meter to define the morphology of the lake.

4.2.5. Heat flux calculations

The heat content in a lake can be obtained directly from temperature data or by the use of the heat budget formulation. The latter is computed as the balance of different sources taking the form

$$Q_H = Q_R - (Q_S + Q_L) \quad \text{(Equation 4.1)}$$

Here, Q_R represents the incoming net radiation in the system, and Q_S and Q_L are outgoing sensible and latent heat, respectively. The resultant Q_H is, therefore, the heat stored in the lake such that positive and negative values represent heat gain and loss, respectively.

To solve partially the previous equation, calculations were made to obtain the seasonal variation of sensible heat flux in Lake Limnoplar. Next, considering that energetic exchange is proportional to temperature gradients just between the water surface and the atmosphere, the boundary bulk formulae (MOMII AND ITO 2008) was used to estimate flux between both layers as follows:

$$Q_S = -\rho_a c_p (T_s - T_z) C_H u_z \quad \text{(Equation 4.2)}$$

where Qs is the sensible heat measured in W m^{-2}, C_p is the heat capacity of air, ρ_a is the density of air (see figure 4.3), and u_Z is the wind-induced friction on the lake surface. Regarding the latter term, the original formulation takes in consideration a turbulent transfer coefficient for heat (C_H); however, it is a direct function of wind speed (V), and often this variable is used directly in the expression:

$$Q_S = -\rho_a c_p (T_s - T_z) V \quad \text{(Equation 4.3)}$$

Calculations were made assuming that the surface temperature in Lake Limnopolar was the same as at 2 m, the depth where the CTD was installed. This assumption was appropriate for summers 2001-02 and 2002-03, as vertical profiles of temperature during these periods showed a nearly isothermal water column. The

required input data of air temperature and wind speed were obtained from the automatic meteorological station (AMS).

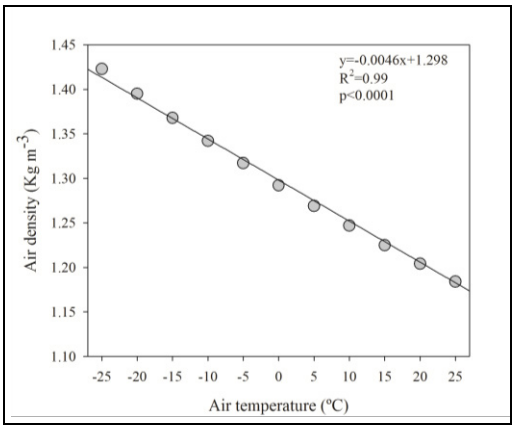

Figure 4.3. *Relation between air density and temperature. The linear relationship between both variables was used to the iterative calculation of the sensible heat using the equation 4.3.*

The flux of solar radiation entering the lake was also derived from data rendered by the AMS. In this case, to obtain data of radiative energy, the values of solar radiation recorded by the AMS (measured in kJ m^{-2} and integrated every 30 min) were divided by 1,800 to obtain kj s^{-1} m^{-2}, which is equivalent to the international system (SI) unity kW m^{-2}.

Differently from previous summers, installation in Lake Limnopolar of a thermistor chain over the summer 2003-04 allowed for different processing in the heat flux calculations. In this case, a thermistor chain was hung on an anchored buoy from 29th December until 12th February to obtain a greater accuracy in the measurements of temporal and spatial temperature variations. The chain was made up of 5 thermistors (Onset Tibdit) placed equidistant along the water column, fixing the position of the uppermost thermistor relative to the ice cap. They were programmed to take a measurement at each 30 min. For all calculations, data were interpolated to acquire 0.25 meter intervals using a least-squares polynomial spline function (STOER AND BULIRSCH 2002). Using these profiles, variations in the volume-weighted heat content of the lake were calculated using equation 4.4 according to HUTCHINSON (1957).

$$H = \sum_{Z=0}^{Z=m} T_z S_h V_z \qquad \text{(Equation 4.4)}$$

where H is the heat content in the lake, z indicates, in meters, the different depths from the surface to maximum depth, T_z is the temperature at depth z, S_h is the specific heat of water, and V_z is the volume of lake for each layer.

Assuming that temperature acts as a passive tracer of the water column disturbance, we used temperatures obtained over the summer of 2003-04 to approximate the vertical heat exchange in the lake. This approximation was made by calculating the vertical eddy diffusivity for heat, K_Z, following the procedures of RAVENS ET AL. (2000). This formulation was originally based on the flux gradient method of JASSBY AND POWELL (1975), which computes the temporal and spatial distribution of a conservative tracer, the heat in our case. Vertical eddy diffusivity can be then obtained by solving the next equation:

$$K_z = \int_{z_4}^{z_1} A_{z'} \frac{\partial T(z',t)}{\partial t} \partial z' \cdot \left[A_z \frac{\partial T_z}{\partial z} \right]^{-1} \qquad \text{(Equation 4.5)}$$

where K_Z is the term for vertical eddy diffusivity. The terms $\delta T_{(z)}/\delta z$ and $\delta T_{(z')}/\delta t$ indicate the vertical gradient and temporal change of temperature at depth z, respectively, and $A_{z'}$ is the cross-sectional area of the lake at depth z'. For these calculations, the water column was divided into 4 slices, each 1 m thick, at time intervals of 30 min, and then the derivative of the function was obtained. Differentiation of this equation yielded the temporal variation of the coefficient K_Z as a function of depth.

Following we include some considerations regarding our procedure for the K_Z estimations. Calculations were restricted to the limits established by the position of thermistors in the water column, that is, from 1 to 5 meters. Heat exchange between boundaries (i.e., ice water and sediment water) was consequently not incorporated in our model. Our calculations are thus devoted to explore diffusivity associated with the pycnocline region. We could have used the soil temperature registered in the AMS to estimate the temperature in the upper layer of ice sheet and then consider the same for the ice layer in contact with lake water column. However, it is disadvantageous to assume that no temperature gradient exists within the ice, as this is not true. Notably, our calculations do not include lateral dispersion, which involves a limitation that we assume and will discuss below. We consider, even so,

that our approximation is sufficient (at least within an order of magnitude) for the purpose of the present work.

Table 4.1. *Summary of parameters and variables used in the calculations of heat fluxes.*

Parameter	Symbol	Unities	Comments and/or used value
Brunt-Väisäla frequency	N^2	s^{-1}	Dependent of *ρw* profiles (LEMMIN 1978)
Density of air	*ρa*	kg m^{-3}	calculated from temperature (Fig. 4.3)
Density of water	*ρw*	kg m^{-3}	calculated in function of temperature and conductivity (FOFONOFF AND MILLARD 1983)
Gravity constant	*g*	m s^{-2}	9.8
Heat capacity of air	*Cp*	J kg^{-1} K^{-1}	1004
Heat content of lake	*H*	kcal m^{-3}	according to HUTCHINSON (1957)
Lake surface	A_Z	m	Obtained from the bathymetric survey
Net heat flux	Q_H	W m^{-2}	Residual of solar and sensible heat fluxes
Photosynthetic radiation	PAR	µmol $(s\ m)^{-2}$	Measured with a luxometer
Sensible heat flux	Q_S	W m^{-2}	Calculation based on the boundary bulk formulation (MOMII AND ITO 2008)
Solar radiation flux	Q_R	W m^{-2}	Calculated with data obtained from the AMS
Specific heat of water	S_H	cal $(g\ ºC)^{-1}$	1
Temperature	*T*	ºC	Obtained with thermometers
Vertical eddy diffusivity	K_Z	m^2 s^{-1}	Estimate based on the flux gradient method (JASSBY AND POWELL 1975; RAVENS ET AL. 2000)
Wind velocity	*V*	m s^{-1}	Data obtained from the AMS

4.2.6. Water column stability calculations

The stability of the water column was numerically estimated by calculating the Brunt-Väisäla frequency profiles (N^2; LEMMIN 1978). Conceptually, it is an index that assesses the effort needed to mix two adjacent water layers. The values were obtained using the following equation:

$$N^2 = \frac{g}{\rho}\left[\frac{\partial \rho w}{\partial z}\right] \qquad \text{(Equation 4.6)}$$

where N^2 is the stability coefficient; $\delta\rho w/\delta z$ is the density gradient as a function of depth, which is a function of two terms, the temperature ($\delta\theta/\delta z$) and specific conductivity ($\delta k_{20}/\delta z$) gradients; and g is acceleration due to gravity. The density values were obtained from temperature and conductivity data acquired at the vertical profiles. For the mathematical details of these calculations, refer to FOFONOFF AND MILLARD (1983). We assume that the pycnocline occurs along the length of the profile at which N^2 maximized. In our case, these observed outcomes are used to compare water column stability between periods of ice cover and open water in the lake.

4.3. Results

4.3.1. Morphometric characteristics of Lake Limnopolar

The bathymetric survey performed in Lake Limnopolar in summer 2002/03 yielded a surface estimation of 22,172.5 m^2, a volume of 58,577.81 m^3, and a mean and maximum depth of 2.64 m and 5.45 m, respectively. The relationships between common morphometric characteristics and the hypsographic and volumetric curves are shown in figure 4.4. Figure 4.5 shows the bathymetric isopleths of the lake. The hypsographic and volumetric curves represent, respectively, the accumulative percentage of surface and volume, which would be found at any depth level of the lake. As shown in figure 4.4, the lake has an ellipsoidal shoreline. It is characterized by a shallow slope of approximately 10 m length through 1.5 m depth. From between 1.5 m through about 3 m, the lake shows a pronounced slope that reaches a plain bottom of around 5 m in depth when the lake is ice-free. The terrain situated in the central part of the lake is somewhat flat and occupies a surface of approximately 12,000 m^2. The relative depth of the lake was 3.24%. This is the maximum depth as a percentage of the lake's mean diameter, and it defines the resistance to mixing (WETZEL AND LIKENS 1991). Compared with most lakes (<2%), this value suggests a moderate resistance to mixing. This resistance could be further favored by the kettle morphology that provides a steeply sloped basin.

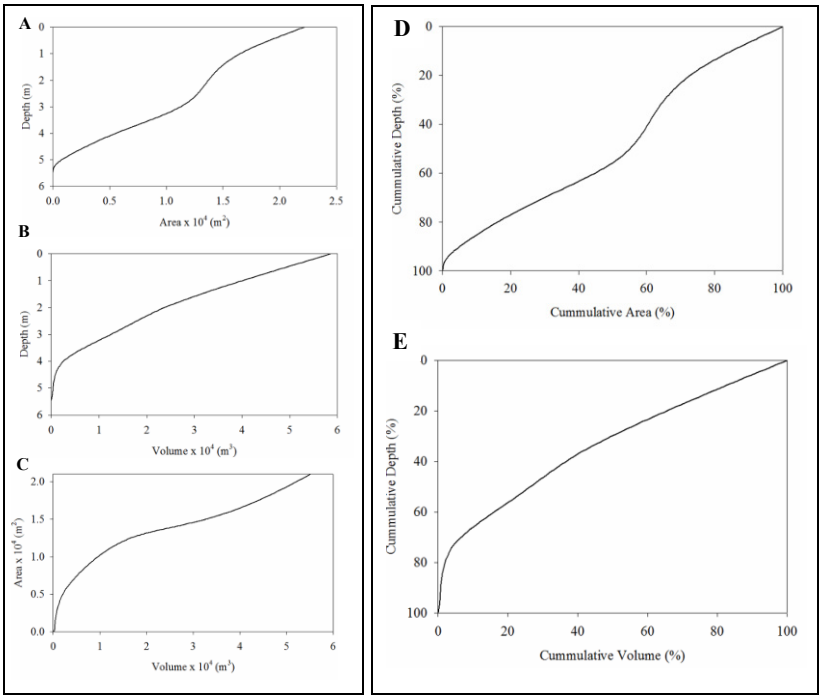

Figure 4.4. *Relationships between the morphometric parameters of Lake Limnopolar obtained with a mapping survey performed at summer 2002-03. A) Depth against area, B) Depth against volume, C) Volume against area D) hypsographic curve and E) volumetric curves.*

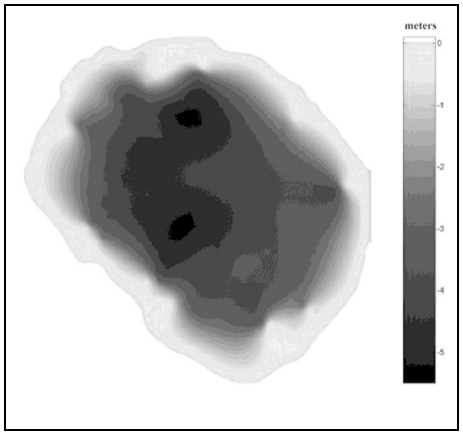

Figure 4.5. *Contour bathymetric map of Lake Limnopolar obtained at February of 2003. The grey scale indicates depth in meters.*

4.3.2. Environmental and ice cover conditions over three summer periods

In the table 4.2 are summarized the main meteorological variables registered by the AMS at Byers Peninsula during three consecutive summers (2001-02, 2002-03, and 2003-04).

Table 4.2 *Summary of mean daily values of meteorological parameters of Peninsula Byres recorded in the AMS at summers 2001-02, 2002-03 and 2003-04.*

	Air Temp. (ºC)	Soil Temp. (ºC)	Water* Temp. (ºC)	Daily Radiation (Kj m^{-2})	Wind speed (km h^{-1})	Max Wind speed (km h^{-1})	Rainfall† (mm)
Summer 2001-02							
average	1.4	1.4	4.1	11,596	26	56	190.4 (66)
max	5	8.2	11.7	29,952	46	104	15.2
min	-1.9	-3.4	-1.8	1,142	9	21	0
median	1.4	1.5	3.8	10,938	26	57	1.5
Summer 2002-03							
average	1.1	1.5	3.7	13,066	22	50	75.1 (35)
max	5.6	9.2	16.4	26,568	52	139	11.4
min	-4.8	-4.1	-0.4	2,963	7	18	0.0
median	1.2	1.7	4.0	12,630	21	46	0.4
Summer 2003-04							
average	0.2	1.0	1.5	12,759	22	46	87.7 (49)
max	4.9	7.7	9.2	29,275	45	90	11.1
min	-8.3	-5.7	-0.4	368	7	17	0.0
median	0.4	1.0	0.7	13,075	22	46	0.3

**Probe installed in Lake Somero*

†Precipitation data are the sum of period (days) indicated in parenthesis

4.3.2.1. Air temperatures

The air temperatures recorded in the automatic meteorological station (AMS) from Juan Carlos I Base (BJCI) and Byers Peninsula correlated significantly (R^2=0.97; $p<0.001$), although Byers Peninsula was, on average, one degree colder. It allowed for an inference of the temperature from Byers Peninsula for periods during no data were recorded. Notably, the air temperature data recorded since 1987 at BJCI in Livingston Island, near the Byers Peninsula, clearly indicated that the summer 2003-04 was the coldest of the 23 years analyzed. Temperatures in the summers 2001-02

and 2002-03 were, on the other hand, in the usual range for this area (Fig. 4.6). Multiple comparison tests provided significant differences between the mean temperatures over summer 2003-04 and the mean for all other summers (it was made excluding observations from November because of the large amount of missing data and using the Bonferroni method with a confidence level of α=0.05). Air temperatures above 0ºC were found 37 days later in summer 2003-04 than in summer 2001-02 and about 20 days later than in summer 2002-03 (Fig. 4.7).

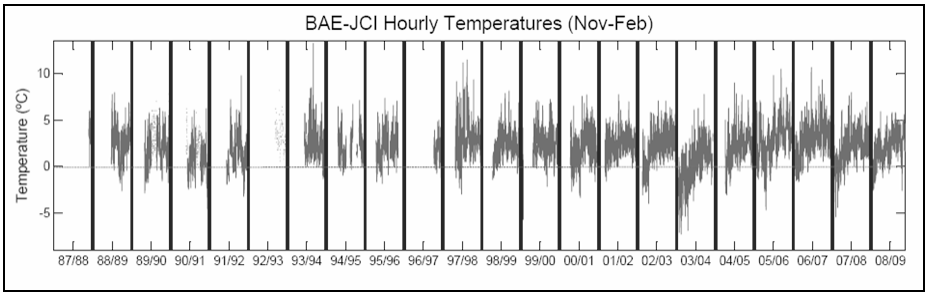

Figure 4.6. *Summer air temperature at Juan Carlos I Polar Base (November-February) from 1987 to 2009.*

Particularly during summer 2003-04, the air temperature oscillated normally between -2/+2 ºC from the beginning to the last week of January. The temperatures increased later, coinciding with a high occurrence of cloudiness and yielding daily variations between 0 and 4 ºC. In addition, a delay in the snow melting during this summer had a buffering effect and affected temperatures near the soil such that they varied at lower levels compared to the other summers (Table 4.2).

4.3.2.2. Wind speed

Summer 2001-02 was on average the windiest, whereas the mean wind velocities for summers 2002-03 and 2003-04 were lower and showed the same behavior. The stronger wind strikes occurred, however, over summer 2002-03. The duration and frequency of the high wind periods was also variable between the summers (Fig. 4.8 and Fig. 4.9). During summer 2001-02 the major wind events lasted a couple of days. In contrast, over summer 2002-03 they occurred regularly at 3-5 days periods. Particularly during summer of 2002-03, velocities during windy periods were up to 7 m s^{-1}, followed by sudden cessations. These velocities showed a decreasing trend

over time through mid January, when higher wind strikes altered this trend. On the other hand, the wind regime during summer 2003-04, at least for the period during which data were available, was characterized by a higher regularity in the occurrence of shorter windy periods (2-3 days) between calm days.

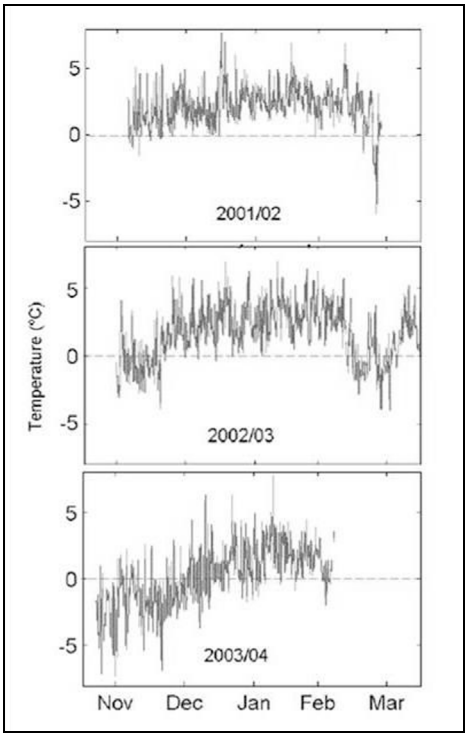

Figure 4.7. *Detailed hourly air temperature from November to April at Juan Carlos I base in the three studied years (2001-02, 2002-03 and 2003-04).*

4.3.2.3. Rainfall and atmospheric nutrients deposition

It is known that strong wind may cause bias (underestimates) in rainfall data acquisition because the occurrence of horizontal precipitation. It is for this reason that rainfall records obtained in our study must be interpreted with caution. Differences were found between all of the studied summers (Fig. 4.10). The total rainfall computed for summer 2001-02 was the highest (190 mm) though these values were recorded over a longer period compared to the others (66 days, from December 8th to January 11th). Thus, the rainfall registered for summers 2002-03 and 2003-04 over 35 (from January 14th to February 17th) and 49 days (from December

28th to February 14th), respectively, was less than half when compared to the first year. This means that, on average, summer 2003-04 was drier relative to the others. Remarkably, the snow compared to liquid precipitation was notably higher over summer 2003-04, which may cause further underestimations.

The atmospheric wet deposition of nutrients was investigated during summer 2003-04. Samples were collected at 6 different dates (Table 4.3), which consisted of rainfall collected from several days (see table 4.3 for details). The two chemical elements analyzed in samples were nitrogen and phosphorus. Nitrogen concentrations varied from 19.2 to 41.5 μg L^{-1}; the highest concentrations coincided with higher rainfall events. Phosphorus also varied notably from 3.3 to 12.9 μg L^{-1}. These variable concentrations also rendered irregular N/P molar ratios. Thus, the ratios ranged from 3.8-25.6; the lower ratios were observed when precipitation was minor (<2 mm).

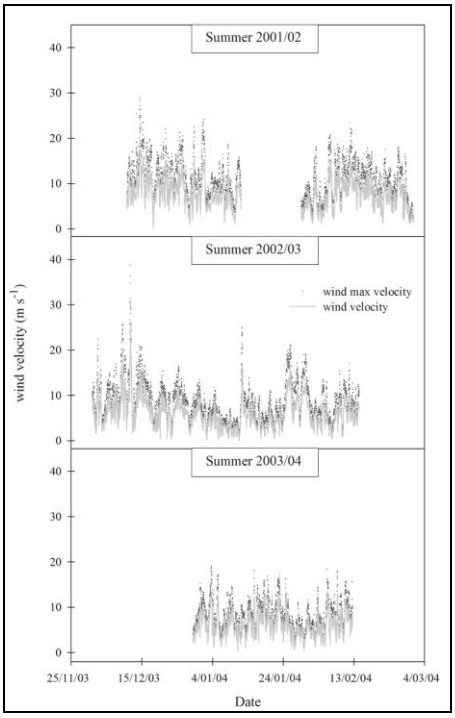

Figure 4.8. *Temporal variations on the average (grey line) and maximum (points) velocities of wind recorded every 30 min in the AMS during the summers 2001-02, 2002-03, and 2003-04. The gaps in the figures are because a malfunctioning of the AMS.*

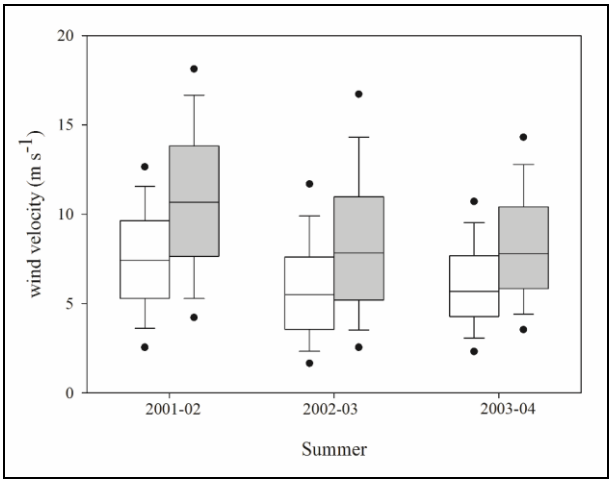

Figure 4.9. *Box-wisker plots showing variation of the average (white box) and maximum (grey box) velocities of wind recorded every 30 min in the AMS during the summers 2001-02, 2002-03, and 2003-04. The box show how both the average and maximum wind velocities distributed at higher values at the first year of the study. By contrast, summer 2003-04 characterized by the quietest winds.*

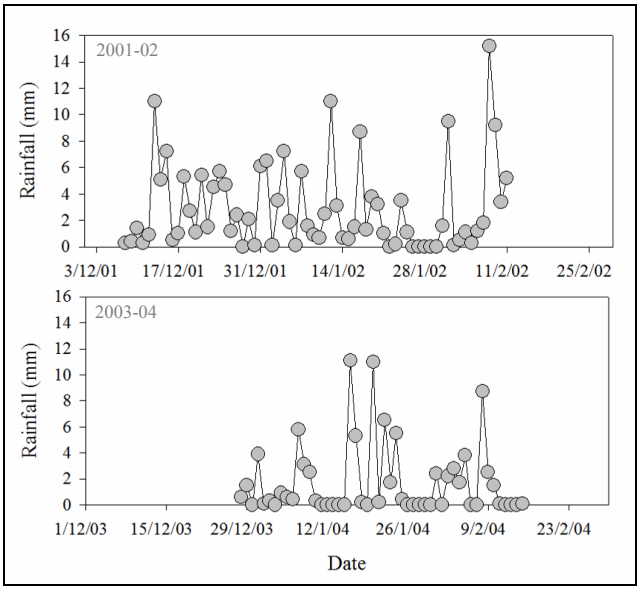

Figure 4.10. *Daily rainfall recorded in Peninsula Byers during summers 2001-02 and 2003-04.*

Table 4.3. *Nutrient concentrations measured in rain samples at summer 2003-04.*

Period	Liters·m^{-2}	µg·L^{-1} N	µg·L^{-1} P	N/P molar ratio
01/9-01/15	2.8	25.12	8.98	6.4
01/15-01/17	1.4	21.67	12.94	3.88
01/17-01/18	11.1	41.45	3.7	25.63
01/21-01/22	5.5	19.86	5.28	8.6
01/22-01/23	0.2	19.24	7.57	5.81
01/23-01/25	3.53	30.94	3.34	21.15
Mean		**26.38**	**6.97**	**11.91**
SD		**8.55**	**3.65**	**9.13**

4.3.2.4. Ice cap dynamics

The continuous record of water temperatures for Lake Somero (water temperatures shown in table 4.2), and the data available for Lake Limnopolar demonstrated differences in water temperature between three years (Fig. 4.11). In general, it was around 0.4º C warmer in 2001-02 than in 2002-03 and around 2.2ºC colder in 2003-04 than in 2002-03. Following the method of CAHILL ET AL. (2005), it was possible to approximate freezing and thawing dates for Lake Limnopolar. As shown in figure 4.11, the estimated ice-free days varied from 96 days in 2001-02 (from December 22nd 2001 to March 27th 2002) to 41 in 2003-04 (from February 11th 2004 to March 23rd 2004). This means that the estimated ice-free period in summer 2001-02 was over twice as long than for summer 2003-04. As a result, summer 2003-04 was the only season in which the evolution of the ice and snow sheet just before the thaw was monitored *in situ*. When the survey began (December 2003), the lake was entirely covered by a cap with approximately 1 m thick of ice and 10 cm of snow. Until mid January, the snow layer uniformly covered the entire lake surface. When the snow began to melt due to the temperature increase, the surface was composed mainly by bare ice (Fig. 4.12). Over the last weeks of January, melting was enhanced and the lake became largely covered with ponded ice. Afterwards, the ice thickness decreased steeply, and by the second week of February the lake was entirely ice-free.

As mentioned in chapter 3, the water level in the lakes from the Byers Peninsula can change dramatically with the freeze and/or thaw processes. This was the case for Lake Limnopolar over summer 2003-04, when it was observed that important variations in water level were associated with the ice dynamics in the outlet. Thus, by early January the ice was detached from the shore, which allowed water ingress through the main inlet. This, assisted by an ice dam at the outlet that

impeded outflow, caused a water level increase from approximately 6 to nearly 8 m. Afterwards, between 12th-13th of January, a sudden break of the dam occurred, drastically reducing the water level around 3 m. After the break, the lake depth was mostly stable at approximately 5 m through completion of the study.

Because of its flat shoreline, these changes in Lake Limnopolar's water level translated to a notable variation of its surface area, shown in figure 4.13. Thus, just before the ice dam broke, the area within the outermost shoreline contour (blue in figure 4.13) was 13,678.56 m^2, which involved that total lake surface, increased to 35,851.06 m^2. If we assume a ground slope of 2 degrees for this additional submerged land, it can be estimated that the additional water increased the lake volume by approximately 71,054 m^3.

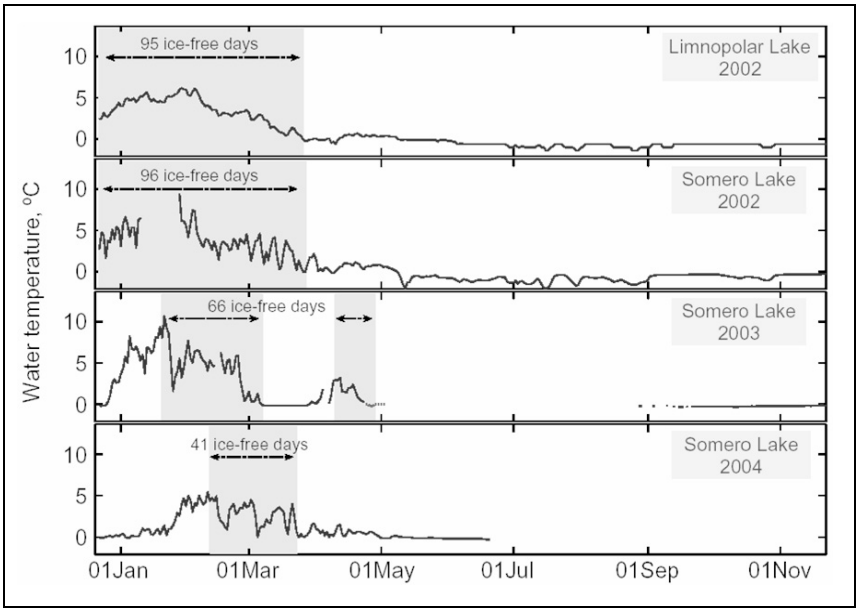

Figure 4.11. *Water temperature (line) and ice-free periods (shaded area) in Lake Somero and Lake Limnopolar (Byers Peninsula) for 2002, 2003 and 2004. The ice melting was considered when visually no ice covered the lake surface. The freezing date was estimated considering the water temperature variation in a given period (see methodological section in this chapter).*

Figure 4.12. *Lake Limnopolar at mid January of 2004. The surface was composed mainly by bare ice, just coinciding with the start of melting period.*

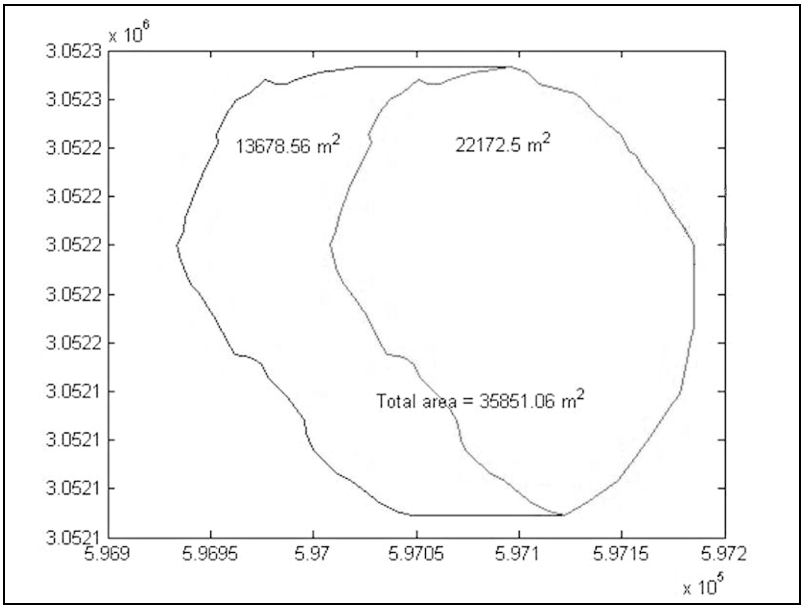

Figure 4.13. *Plot showing the increase in surface suffered by Lake Limnopolar during a short period at summer 2003/04. It happened because when ice melting started the stream inlet began to flow into the lake before that the ice dam of the outlet thawed.*

4.3.3. Optical characteristics of the water column

The light (PAR) profile measurements carried out for summer 2002 (mid January) in Lake Limnopolar, with any ice-cap, revealed an optically homogeneous and highly transparent water column (k_{PAR}=0.35 m^{-1} ± 0.15SD). As a result, approximately 25% of the surface incident light reached the bottom of lake during this period. Profiles for summer 2002-03 were quite similar, showing k_{PAR} values of 0.30 m^{-1} ± 0.10SD and 0.20 m^{-1} ± 0.12SD for mid January and February, respectively. During this period, an increase in the light reaching the lake bottom was observed, 20% to 45% of the surface irradiance.

Figure 4.14 shows the logarithmic plots of PAR irradiance as a function of depth during summer 2003-04. The presence of an ice and snow cover, which in this summer lasted until February, largely affected the optical characteristics of the water column, demonstrated by the evolution of k_{PAR} during this summer (Fig. 4.14). In general, attenuation of PAR decreased with time. From the last December to the last January, the optical characteristics of the ice shifted from low to high transparency due to the snow and ice melting. Thus, when the ice and snow sheet was thickest, less than 3% of the surface irradiance reached the bottom of lake, whereas at the beginning of snow melting, the transparency increased (over 7% of the surface irradiance reached the lake bottom). In contrast, transparency values in the water column decreased gradually and in parallel to reduced thickness of the ice and snow cap. On the other hand, when lake was ice-free, similar values to the previous two years were observed (around 20% of surface irradiance reached the lake bottom).

4.3.4. Thermohaline structure and stability of the water column

The thermal characteristics of the water column in Lake Limnopolar differed notably depending on the ice condition (Fig. 4.15). For all years, the water column during ice-free periods was almost isothermal. Over summer 2001-02 the maximum water temperature at 2 m depth reached 6.4 ºC. On the other hand, for summers 2002-03 and 2003-04 the maximum temperatures were 7.2 ºC and 4.2 ºC, respectively. It is also remarkable that the water temperature remained between 2 and 4ºC colder over the entire summer 2003-04 than in the other two studied years.

Similar to temperature, conductivity profiles for summer 2001-02 were characterized by a uniform distribution (Fig. 4.15). Variations over time were also narrow. Mean values over the water column increased gradually from 52 to 77 µS cm^{-1}. During summer 2002-03, no important variations were observed in the

conductivity profiles, though the mean was somewhat higher in mid January (76 µS cm^{-1}) compared to mid February (60 µS cm^{-1}). In late December of summer 2003-04, the values for almost the entire water column were also quite homogeneous, although higher conductivities (137.9 µS cm^{-1}) were observed near the bottom. By mid January, the conductivity gradient was more pronounced, from approximately 2 m to the bottom, when circulation in the lake increased and the inlet water flowed into the lake. At this time, conductivities ranged from 12.3-204.4 µS cm^{-1}. This vertical gradient remained nearly constant until thawing at the end of January, when the largest differences in conductivity were observed between the surface of the lake and the bottom. When ice melted completely in February, conductivity became constant with depth.

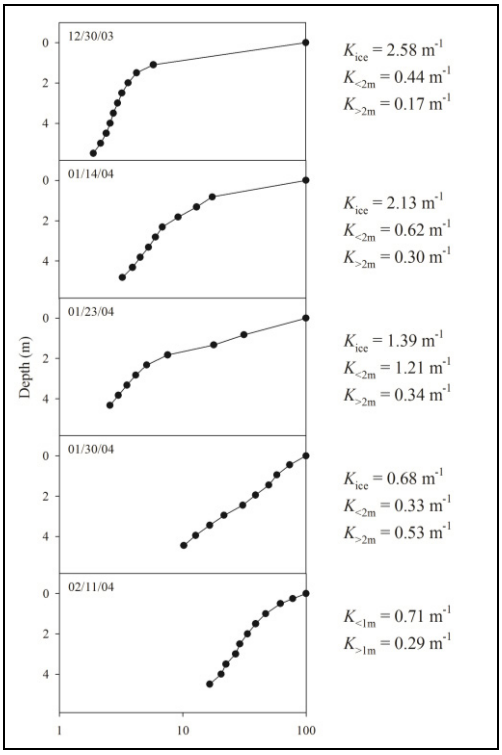

Figure 4.14. *Changes of extinction coefficient (k) of PAR irradiance of the ice cap and in the water column during summer 2003/2004.*

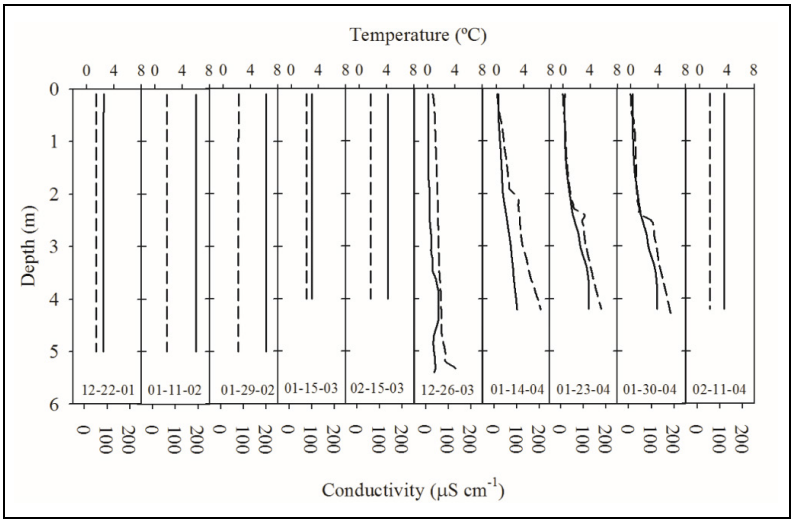

Figure 4.15. *Vertical profiles of temperature (solid line) and conductivity (dashed line) in Lake Limnopolar used to make stability calculations at different dates of summers 2001-02, 2002-03 and 2003-04.*

The vertical profiles of stability, expressed as Brünt-Väissäla frequencies (N^2), obtained from the three summers are depicted in figure 4.16. Both temperature and conductivity profiles determined the shapes observed. The N^2 values for summer 2001-02 were consistently below $1x10^{-4}$ s^{-1} throughout the entire water column, indicating a complete mixing, even when the lake was still partially covered by ice (22nd December). Only on 29th January was a slight increase in surface water observed as a consequence of somewhat higher temperatures. In contrast, a short, inverse stratification period was established over January 2004. Just before this period, in late December, only near-bottom and surface stratification peaks were observed, due to the slight increase in conductivity and the onset of inverse thermal stratification. Nevertheless, as observed in figure 4.16, the pycnocline was only obvious at 2 m during mid January. This pycnocline deepened slightly, settling down to 2.5 m depth at the end of January. Afterwards, by mid February, the ice cover thawed completely and holomixis occurred. As a result, during the pycnocline period, two layers of the water column were isolated, namely, an upper mixing layer (varying between 2 and 2.5 m depth) and a lower, fairly stagnant layer, which can be considered as a epilimnion and a hypolimnion, respectively.

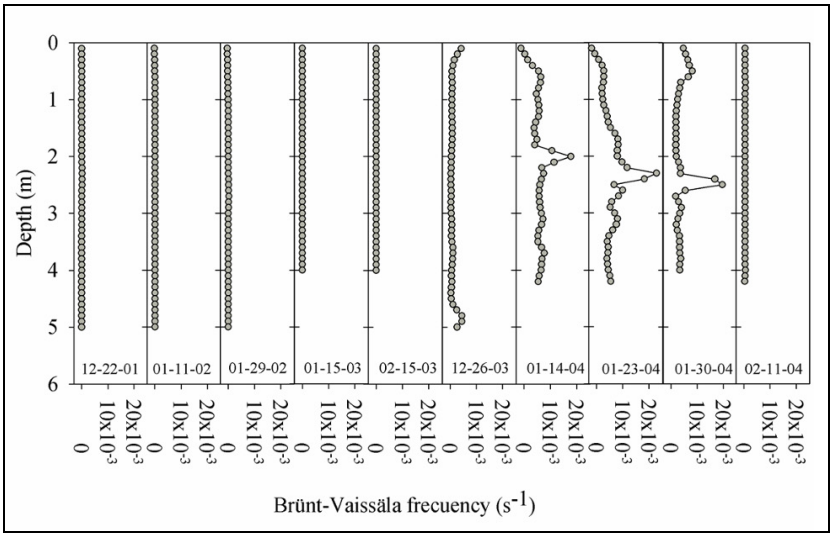

Figure 4.16. *Vertical stability in the water column in Lake Limnopolar at different dates expressed as the vertical profiles of Brünt-Väissäla frequency*

In addition to the above profiles, a CTD probe was positioned at 2 meters depth to continuously register temperature with a frequency of 30 min. This procedure was followed in the same way for the three summers, from 28-Dec-2001 to 11-Jan-2002, from 16 to 31-Jan-2003, and from 30-Dec-2003 to 23-Jan-2004. As observed in figures 4.17 and 4.18 for summers 2001-02 and 2002-03, respectively, the occurrence of temperature oscillations in the water column was verified, suggesting either thermocline tilting or internal seiches. To determine what forced this tilting of temperature, the temperature coupling to both wind and solar radiation was analyzed via cross-correlation analysis. The cross-correlation data that we present here are those obtained for interval time of a day. Additional analyses were made to determine whether time periods over a day also reported a periodic auto-correlation, but no significant signal was observed between either pair of variables. Further, given that the cross-covariance might be not necessarily symmetric about zero, both positive and negative lags were computed for this timeframe.

During summers 2001-02 and 2002-03, over the ice-free periods, water temperature at two meters depth changed in a quasi-regular oscillation that matched well with the dial cycle of solar radiation and air temperature, suggesting a pattern of a diurnal slight stratification and mixing. As demonstrated by cross-correlation analysis (Fig. 4.19), the correlation was somewhat higher for 2001-02 compared with the following summer. This analysis furthermore demonstrated the way by

which the water temperature of lake lagged behind the causal variables. Thus, the cross-correlation coefficients between solar radiation and water temperature were generally moderate and peaked positively and negatively at lags 6 and -6 hours, respectively. This closely coincided with that observed for the air temperature. There was not autocorrelation, however, at -6 hour lags for summer 2002-03. Lags also varied compared to summer 2001-02 for the rates of water temperature change. Thus, coefficients in this case were even higher for periods of 0 and 12 hours in a positive and negative way, respectively.

In contrast, the temperature fluctuation exhibited less covariance with the wind velocity, as observed in the autocorrelograms (Fig. 4.19). However, it was detected as a negative effect that lagged 19 and 12 hours for summers 2001-02 and 2002-03, respectively. For summer 2001-02, this negative effect was moderate and affected temperature rather than its rate of variation. In contrast, the effect was weaker for summer 2002-03, although in this case both temperature and its variation rate were similarly influenced. Additionally, for summer 2001-02 in particular, a moderate, positive effect of wind velocity on water temperature was observed, which lagged -18.5 hours.

Notably, wind velocities for both years exhibited some structure other than periodic that dramatically affected the temperature evolution, indicating the existence of a larger scale coherent wind force. For instance, as shown in figure 4.17, three clear discontinuities occur in temperature data over summer 2001-02 that match with stronger wind events on days 2, 5 and 12. As observed in the same figure for the rate of temperature change, the increases were largest just before these events. In the case of summer 2002-03, the changes in temperature induced by wind were weaker, as mentioned before, such that a constant increase of temperature took place during the first 8 days (Fig. 4.18). However, an increase of wind velocity of around 3 fold during days 9 and 10 triggered water column mixing and, as a result, a notable drop in the water temperature.

The thermal regime for summer 2003-04 differed notably from the two preceding years because an ice cap was present in the lake. As a consequence of this isolation from the atmospheric forcing, no significant correlations were observed between water temperature and environmental variables. However, there was a weak, perceptible positive co-variation between water temperature variation and both solar irradiance and wind velocity (Fig. 4.20). However, the coefficients observed for wind velocity were the residuals of its covariance with solar radiation, clearly indicating that no direct causality existed between wind velocity and water temperature. A noteworthy outcome observed from this summer was that the cross-

correlation between solar radiation and water temperature differed in phase when compared to previous summers. Higher coefficients, both positive and negative, occurred at lags -2 and 10 hours, which implies that the target variable's response was delayed 2 hours when lake was ice covered compared with that observed for the ice-free periods.

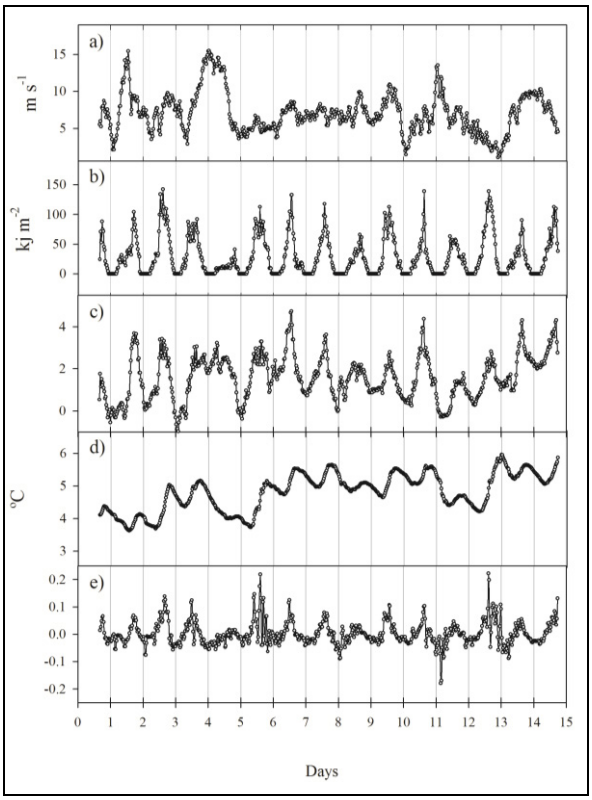

Figure 4.17. *Time-series of environmental parameters and thermal characteristics of Lake Limonopolar at summer 2001-02. Plots are wind velocity (a), solar radiation (b), air temperature (c), water temperature in lake at two meters depth (d), and variation of temperature in lake at two meters depth (d). Data were collected from 28th December to 11th January.*

The time courses for both radiative heat (gained by the lake) and sensible heat (lost by the lake) over summers 2001-02 and 2002-03 are shown in figures 4.21 and 4.22, respectively. The residual energy stored in the lake, indicated with a red line in these figures, was partially resolved by subtracting sensible heat from radiative flux. As observed for two summers, the level of energy stored into the lake

followed the diurnal cycle upon radiation balance, that is, maximum values occurred in the middle of the day and minimum values during the night. In contrast, wind cools down the lake surface during the afternoon hours as observed by the increase of sensible heat flux going to the lake (i.e., negative values). This was a common trend for the two summers; though heat fluxes out of the lake were, on average, higher for summer 2001-02. The highest episodic sensible heat losses occurred over summer 2002-03, coinciding with lower solar irradiation. As observed in figure 4.22, this implied a negative balance of heat, close to -100 W m^{-2}

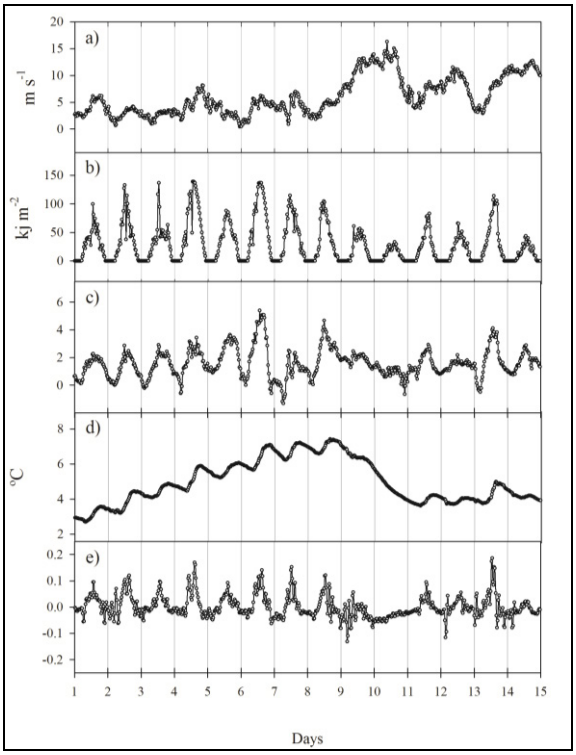

Figure 4.18. *Time-series of environmental parameters and thermal characteristics of Lake Limonopolar at summer 2002-03. Plots are wind velocity (a), solar radiation (b), air temperature (c), water temperature in lake at two meters depth (d), and variation of temperature in lake at two meters depth (d). Data were collected from 16th to 31st January.*

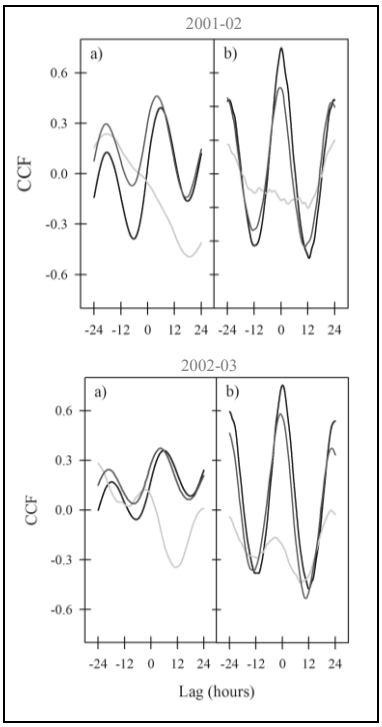

Figure 4.19. *Cross-correlations coefficients (CCF) between environmental parameters and temperature (a) and temperature variation (b) at two meters depth in Lake Limnopolar at summers 2001-02 (up) and 2002-03 (below). Environmental parameters are solar radiation (black), wind velocity (clear grey), and air temperature (dark grey).*

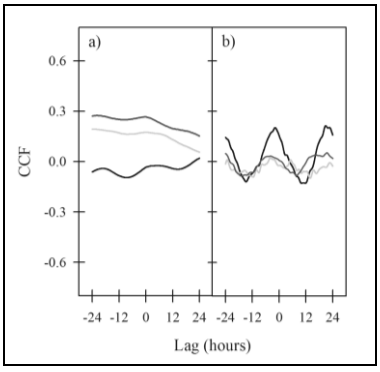

Figure 4.20. *Cross-correlations coefficients (CCF) between environmental parameters and temperature (a) and temperature variation (b) at two meters depth in Lake Limnopolar at summer 2003-04. Environmental parameters are solar radiation (black), wind velocity (clear grey), and air temperature (dark grey).*

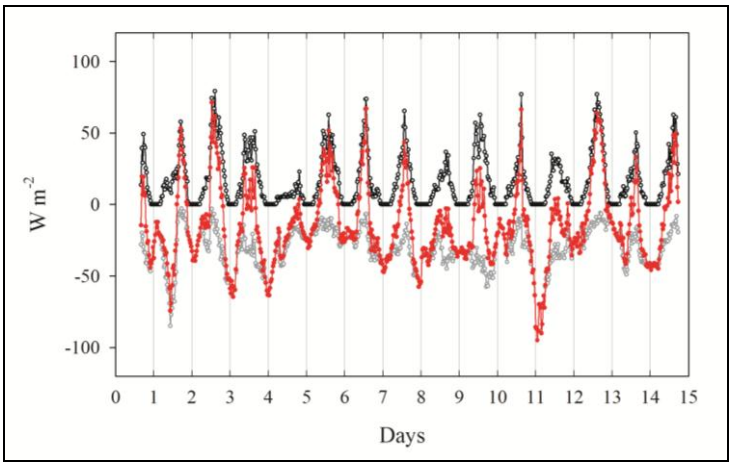

Figure 4.21. *Heat and radiation fluxes at the water–air interface of Lake Limnopolar as calculated for the period from 12-28-01 to 01-11-02 based on 30 min interval measurements. The series of heat fluxes are radiation (black), sensible heat (grey) and the balance among them (red).*

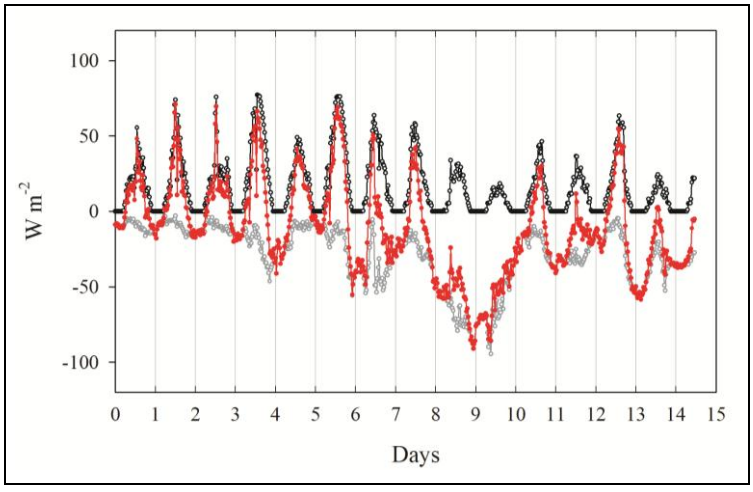

Figure 4.22. *Heat and radiation fluxes at the water–air interface of Lake Limnopolar as calculated for the period 01-16-03 to 01-31-03 based on 30 min interval measurements. The series of heat fluxes are radiation (black), sensible heat (grey) and the balance between them (red).*

4.3.5. Heat content variation and thermal diffusivity in the lake during summer 2003-04

Along the 2003-04 summer, the thermal regime in Lake Limnopolar was more accurately measured using a chain of thermisthors. This installation provided a temporal resolution as high as one temperature value per 30 minutes (Fig. 4.23). When measurements began at the end of December with the lake still covered by ice, the water column was almost thermally homogenous, with slightly warmer temperatures (~1ºC) at the bottom. From mid January, an evident inverse stratification built up. During this period, the colder water, close to 0ºC, was at the surface just under the ice, whereas the deepest layers were near 4ºC. Thus, through this timeframe, mainly when the ice was thinner and circulation increased in the lake, convective plumes migrated gradually through the water column, transporting heat downwards. This resulted in the progressive heating of the deep layers, beginning around mid January and continuing through the first week of February. Finally, this gradual heating generated a temperature gradient between the surface and deep layers of approximately 3.5 ºC. This thermal discontinuity disappeared when the lake was entirely free of ice, which was accompanied by temperature fluctuations that followed the diurnal cycle. These changes in the thermal regime of the lake are also illustrated in the range of daily temperature variations, shown in figure 4.24. Here, it is observed that marked temperature changes occurred when the dam fractured over mid January, though mostly after the ice cover was thawed. This is in contrast with both the observed temperature changes at the end of December until the outlet opened, and those during mid January, when the water column maintained higher thermal stability.

During the austral summer, as a general trend, daily global radiation decreased with time due, in part, to the decrease in the illuminated hours of the day. Another general trend was the occurrence of cloudy days, which were more frequent from the end of January. The net radiation arriving at the lake surface was estimated based on profiles of PAR and the regime of solar radiation obtained from the AMS. Some previous considerations were made for calculations as follow. Given that the values of global radiation rendered by the AMS were in kJ m^{-2}, they were transformed to calories (1 calorie equals 4.1868 joules). Following, we applied the percentages of light extinction obtained from the underneath PAR profiles to the global solar radiation data obtained from the AMS (Fig. 4.25). The percentage of light extinction for the different sampling dates was obtained using linear interpolation. Our calculations assume that the ice is an optically homogenous layer.

We recognize the limitation of this procedure given that bands of ultraviolet and infrared radiation are less available below the ice and selectively absorbed by water.

As shown in figure 4.26, the lake generally gained heat as summer advanced, though the ice cover condition and some episodic events altered this trend. Hence, from last days of December to the second week of January, the heat content in lake remained stable at 100 Kcal m^{-3}. The daily variation of heat content during this period was also the lowest for the overall times studied (Fig. 4.27). A slight increase in heat content occurred on the 6th of January, but it was lost again over the next few days. A more notable increase in the stored heat started just after this period, when the lake outlet opened (10th-13th Jan). Over the next 10 days, the lake ceased to gain heat appreciably despite the increase in incident solar radiation. This situation remained somewhat stable until the last week of January. At this point, the heat content in the lake began to increase more steeply until it reached the highest values observed during the ice cover period (~650 Kcal m^{-3}). This heat increase stored in the lake likely helped to melt the ice. As the ice melted, a notable decline in the heat content of the lake took place, and approximately 200 cal cm^{-3} were lost during the first days of February. This coincided with a decline in solar radiation. Therefore, although the light coming into the lake at this time was not impeded by ice during this time, the solar heating throughout the lake was on the order of that observed during January. Later, when the heat provided by solar radiation increased again, the lake started to gain heat until it reached values close to 800 cal cm^{-3}.

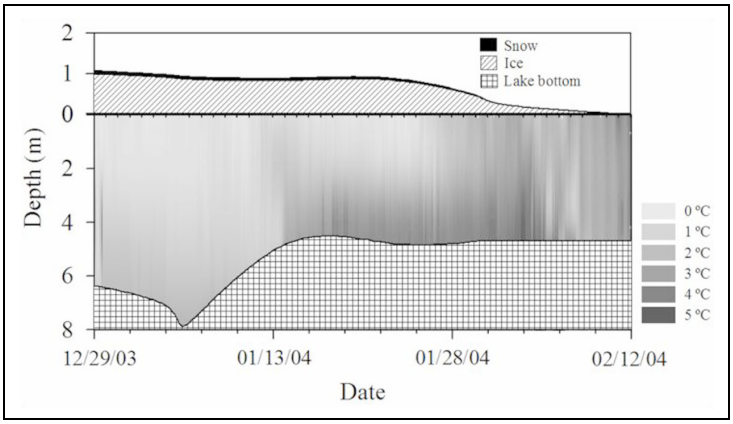

Figure 4.23. *Time-course of temperature regime in Lake Limnopolar during the summer 2003/2004 obtained from data of the thermistors chain.*

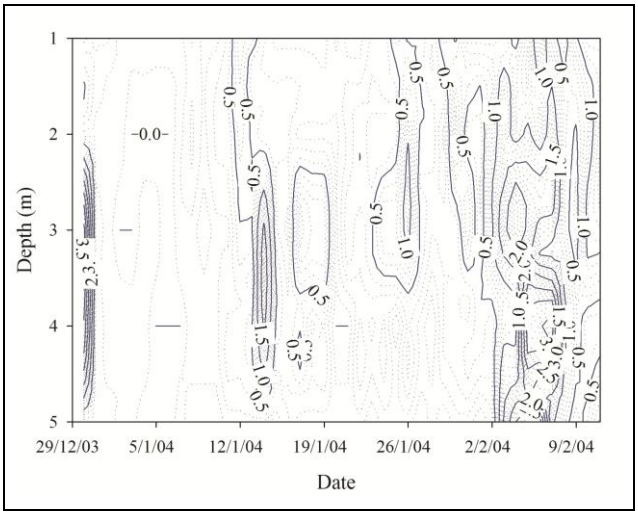

Figure 4.24. *Isopleths of daily temperature variation in the water column of Lake Limnopolar during the summer 2003/2004.*

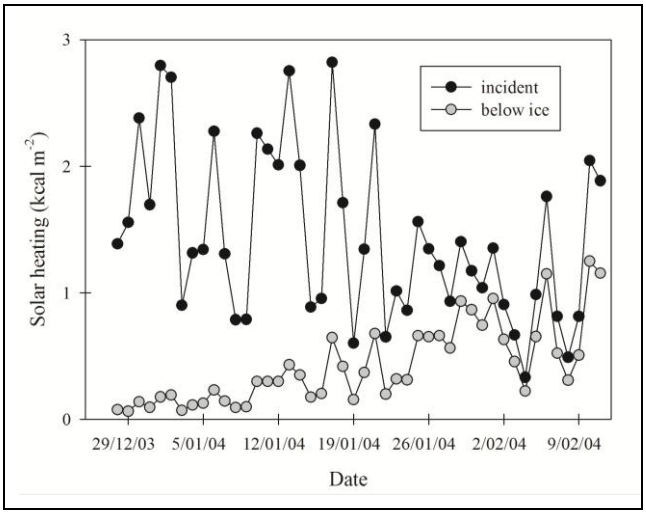

Figure 4.25. *Variations in the incident and below ice daily solar heating in Lake Limnopolar during summer 2003-04. The amount of light absorbed trough the ice was estimated based on the observed PAR attenuation by ice in the regular profiles performed in the lake. Data between different sampling dates were obtained by linear interpolation of data from the AMS.*

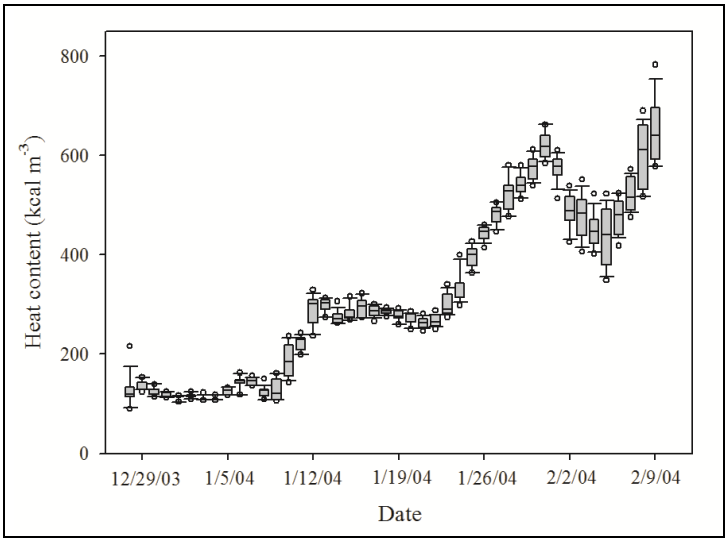

Figure 4.26. *Box plots showing the evolution of the heat stored daily in Lake Limnopolar during summer 2003-04. The solid line inside the boxes indicates the median, the end of the boxes correspond to the interquartile range and the whiskers to 5% and 95% percentiles.*

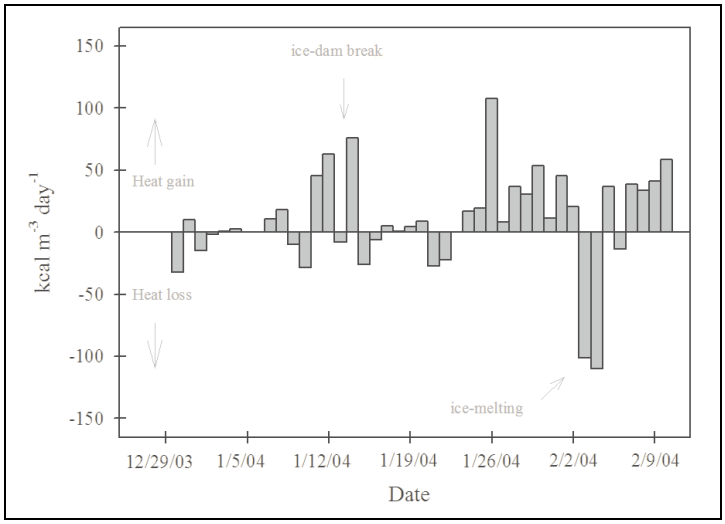

Figure 4.27. *Daily heating rates in Lake Limnopolar during summer 2003-04.*

Using the continuous profiles of temperature recorded for summer 2003-04 we can estimate the thermal diffusion (K_Z) in the water column. This was estimated by evaluating the first (time) and second (space) moments of the temperature changes. Given that both conductive and non-conductive heat transfer may occur, as discussed in more detail below, it may be most appropriate to consider the K_Z as "apparent" or "effective" thermal diffusivity. Otherwise, large differences were observed depending on the way in which the data set was calculated. If a least-squares polynomial interpolation is applied to the temperature data, then it produces incoherent results, indicating the need to use only the real data set rendered by thermisthors.

As shown in figures 4.28 and 4.29, the variation of the K_Z coefficient estimated for Lake Limnopolar is a function of both depth and time. Table 4.4 and figure 4.30 show the means and dispersion for the measurements at different layers. During the more quiescent period, just before the outlet damn break, K_Z fell within the range of 0.8-3.3 x 10^{-5} m^2 s^{-1}. The higher values during this period were observed in December between 2 and 3 meters, and then they dropped progressively at these depths as increases occurred at the bottom. The deepening of this maximum of K_Z stopped by mid January, coinciding with the opening of the outlet.

After the partial lake drainage, values of K_Z were at maximum, just above the pycnocline, and then plumes transporting heat downward were formed. Once the pycnocline was defined near the midpoint of the water column, more notable differences appeared for K_Z between the upper and lower layers and demonstrated a clear dependence on water column stratification. K_Z in the mixing layer were up to 3 x 10^{-5} m^2 s^{-1}, with maximum values just over the maximum of the N^2 profile. In contrast, during this period apparent eddy diffusion below the pycnocline showed basal values around 1 x10^{-5} m^2 s^{-1}, the lowest observed during the entire period. The turbulence generated over this period via the downward transport of heat from the upper strata eroded the pycnocline, thus increasing the depth of the mixing layer. This phenomenon is exemplified in figure 4.29, in which sinking in the N^2 peak is observed from mid to late January (red points in the plot).

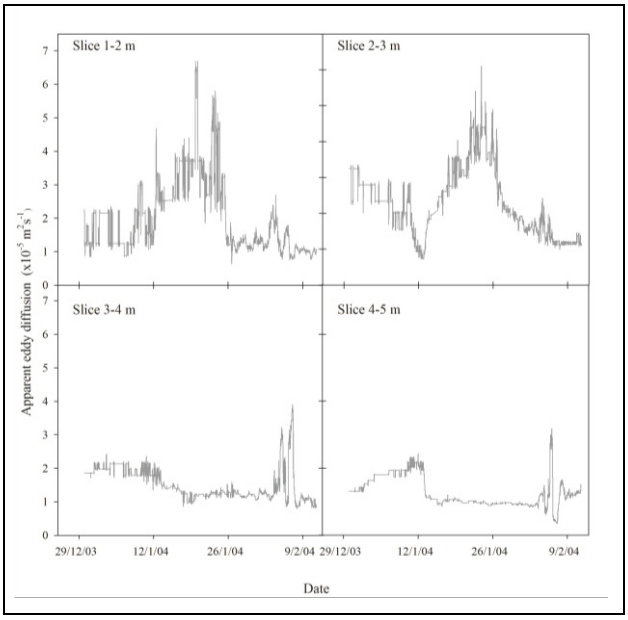

Figure 4.28. *Time-course evolution of the apparent eddy diffusivity at different layers of lake. The slices between 1 and 3 meters and between 3 and 5 were respectively over and below the pycnoclyne.*

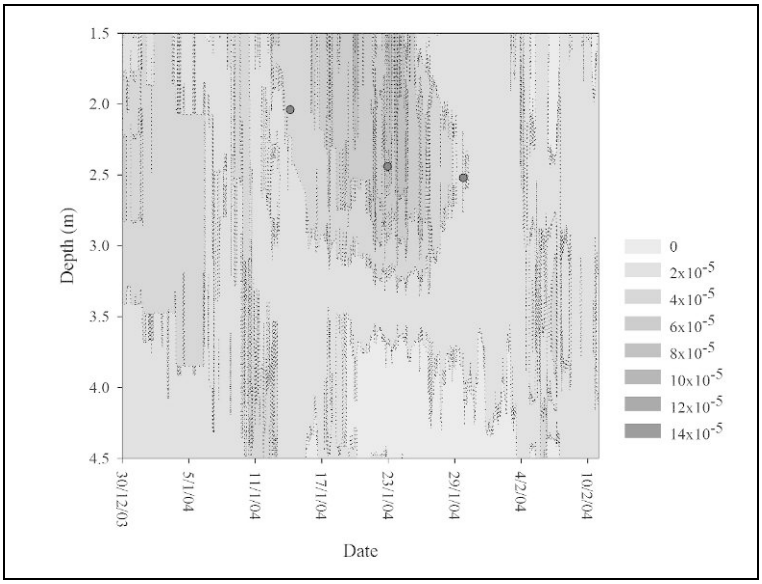

Figure 4.29. *Isopleths of eddy diffusivity (m^2 s^{-1}) in the portion of the water column from 1 to 4 of Lake Limnopolar. Points indicate the depth at which maximum stability occurred.*

Table 4.4. *Mean and range of variation for the apparent coefficients of thermal diffusion (Kz) obtained at different layers of water column during summer 2003-04.*

Layer		n	Mean	SD	Minimum	Maximum
1-2 m	Above the pycnocline	2046	2.00×10^{-5}	1.14×10^{-5}	6.31×10^{-6}	7.91×10^{-5}
2-3 m		2046	2.30×10^{-5}	9.34×10^{-5}	7.49×10^{-6}	5.87×10^{-5}
3-4 m	Below the pycnocline	2046	1.53×10^{-5}	4.82×10^{-5}	8.05×10^{-6}	3.87×10^{-5}
4-5 m		2046	1.29×10^{-5}	4.50×10^{-5}	3.42×10^{-6}	3.18×10^{-5}

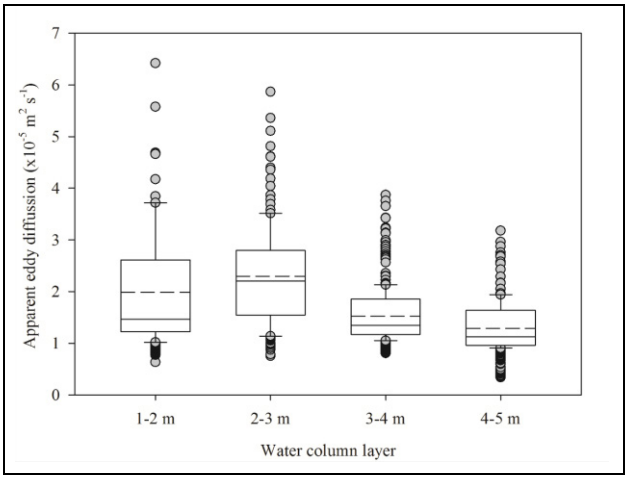

Figure 4.30. *Box-wisker plots showing the distributions of the calculated eddy diffusion coefficients (K_Z) obtained for different layers of water column in Lake Limnopolar at summer 2003/04. The solid and dashed lines inside the boxes indicates median and mean respectively, the end of the boxes correspond to the interquartile range and the whiskers to 5% and 95% percentiles.*

4.3.6. Dissolved and particulate nutrient dynamics

Both inorganic and particulate nutrient concentrations varied among and within summer periods, as shown by figure 4.31. Soluble reactive phosphorus (SRP) concentrations varied among different years from undetectable levels to around 0.1 µM. During summers 2001-02 and 2003-04, concentrations generally decreased as the season advanced, with higher concentrations occurring during ice cover or at the onset of melting. For summer 2003-04 in particular, this trend was more accentuated in the bottom layers. On the other hand, no important differences were observed within summer 2002-03; though, a slight increase was observed at the end of the summer period (0.05 µM).

Dissolved inorganic nitrogen (DIN) was low over the three investigated summers, typically below 2 µM, and ammonium was the dominant form. In general and despite differences in the ice condition among years, higher concentrations of DIN occurred during the first weeks of January and declined progressively until they reached minimum values in February. The concentration of nitrate plus nitrite (NO_x) in the surface water at the beginning of the thaw in summers of 2001-02 and 2003-04 was lower than 0.2 µM. This concentration reached up to 0.3 µM in 2001-02, up to 0.6 µM in 2003-04, and then progressively dropped to reach extremely low concentration values in the last sampling event of each of these years. Similarly to what was observed for SRP over summer 2003-04, NO_x concentrations at the bottom were highest in December and fell sharply in January. The evolution of NH_4^+ concentrations mimicked closely those displayed by NO_x, though one order of magnitude higher. Further, they were notably high just before the ice thaw and lower at the end of January.

Trends in both particulate nitrogen (PN) and phosphorus (PP) also differed substantially between different summers. The PN concentration was stable (ranging between 7.2 and 9.7 µM) in 2001-02, and in contrast, a 4 fold increase for the period near the ice thaw followed by a marked decrease was observed in 2003-04 at two depths. PP concentrations followed similar trends as for PN in 2003-04, averaging 0.49(±0.14SD) and 0.48(±0.09SD) µM at the surface and at lower depths, respectively. In contrast, they decreased over summer 2001-02, giving rise to a marked increase in the TN/TP ratio, which varied broadly between 22.2 and 94.3 in 2001-02. This ratio was more stable in 2003-04, with higher values (31.49 and 38.97 at the surface and bottom, respectively) when ice began to melt.

4.3.7. Dynamics of chromophoric dissolved organic matter (CDOM) during summer 2003-04

The occurrence of the optically active fraction of dissolved organic matter (i.e., chromophoric dissolved organic matter: CDOM) was determined for summer 2003-04. In all samples analyzed, from late December to mid February, two maximal fluorescence signals at the excitation/emission pairs 240nm/388nm and 290nm/395nm were regularly detected (Fig. 4.32). The temporal evolution of these fluorescence signals is depicted in figure 4.33, which we can ascribe to humic acid substances, analogous to the fluorophores A and C described by COLE ET AL. (1996). Until mid January both signals showed similar trends. However, beginning Jan 23rd, the two peaks showed independent trends as a function of time and depth.

Component A increased mostly as a function of time, reaching higher values at two depths at the end of January, shown in figure 4.33. In contrast, component C only showed significant increases at the bottom of the lake, with higher values occurring a short time before. As a remarkable trend, a displacement in the position of the peak C to shorter wavelenghts of emission was observed just after this maximum on January 30th (Fig. 4.34), likely indicating a change in the chemical composition or structure of these chromophoric substances.

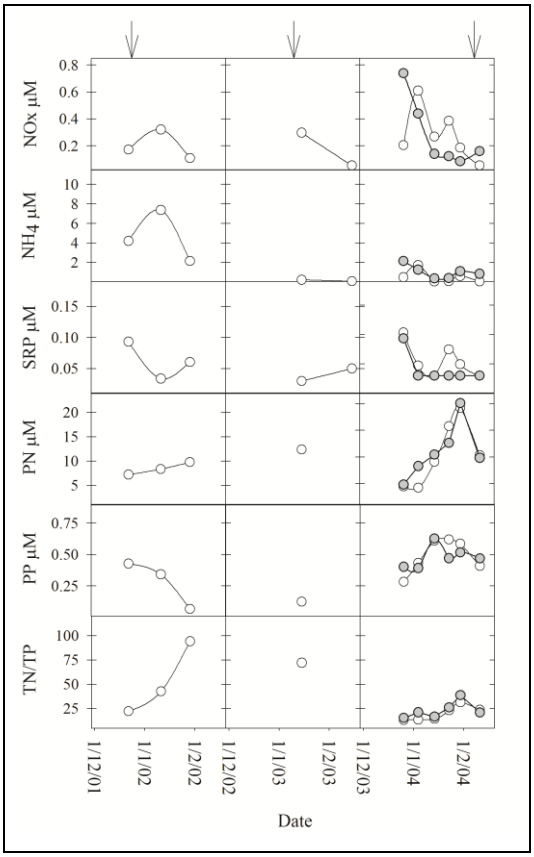

Figure 4.31. *Changes of major nutrient concentrations at surface (white circles) and bottom (grey circles) waters along time in Lake Limnopolar in the three summer periods studied. Arrows indicate the time of ice melting.*

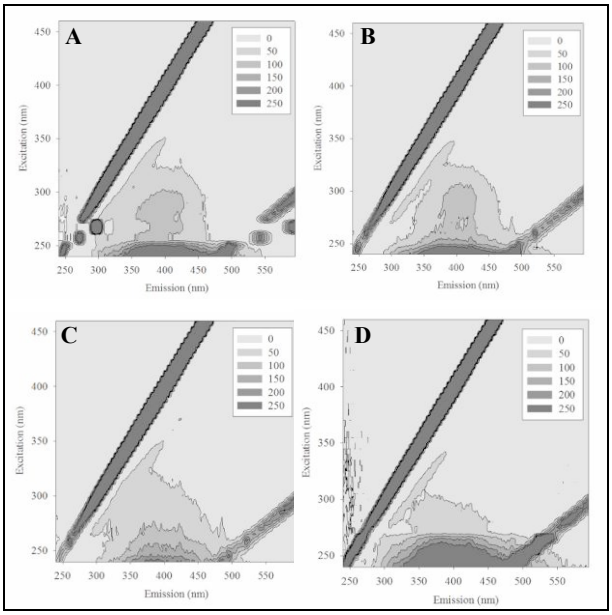

Figure 4.32. *CDOM signature represented by the Excitation/Emission matrix of 0.2 µm filtered samples from surface waters of Lake Limnopolar at summer 2003-04. The date of samples are A) 26-Dec, B) 14-Jan, C) 23-Jan, and D) 30-Jan. The grey-scale legend indicates fluorescence unities.*

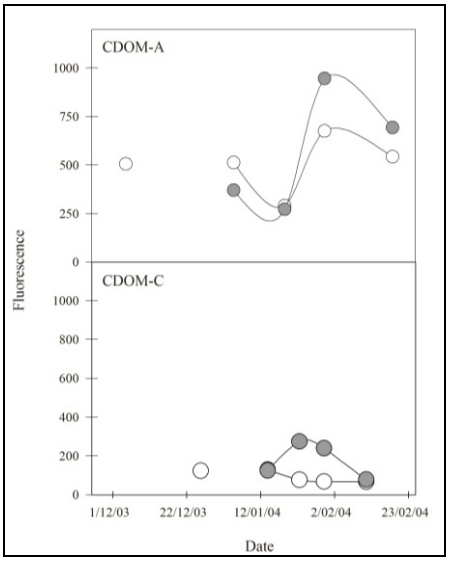

Figure 4.33. *Changes of major CDOM components along time in Lake Limnopolar during summer 2003-04 at surface (white circles) and bottom (grey circles) waters.*

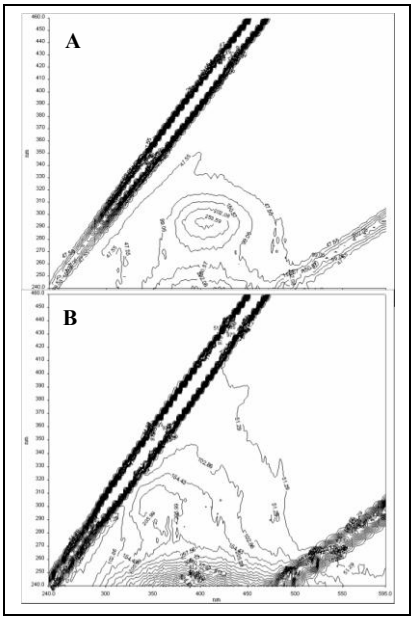

Figure 4.34. *Some three-dimensional excitation-emission matrices (EEMs) obtained from the deep layers in Lake Limnopolar at 23th Jan (A) and 30rd Jan (B).*

4.4. Discussion

Studies on variations in ecosystem function driven by meteorological conditions are of great interest in building global change models. In this chapter, we present data on meteorologically driven ecosystem variation over a short period of time (the summer period of 3 consecutive years). These results demonstrate the wide natural variation of these extremely sensitive non-marine aquatic ecosystems and may operate as a baseline for future climate change studies in regions where the changes are more pronounced such as the maritime Antarctica (QUAYLE ET AL. 2002).

The area studied here shows mild summers, with average temperatures slightly above 0ºC (Fig. 4.6). This proximity to the freezing point makes the aquatic ecosystems of this region very vulnerable to slight temperature changes, which can trigger remarkably longer or shorter ice-free periods, as ice duration on a lake is 60-70% a function of the air temperature (PALECKY AND BARRY 1986, LIVINGSTONE 1997). This effect is clearly illustrated in figure 4.11, showing that an earlier decrease in temperature for February 2003 triggered a lake water freeze one month earlier than is typical for this area. However, weeks later the temperature increased again and promoted a new thaw.

Summer 2001-02 was warmer and windier relative to the others. Summer 2002-03 was only a bit colder than the previous summer, whereas summer 2003-04 was considerably colder (more than 1ºC colder for the summer daily average than in 2001-02). This variation influenced notably the length of the ice-free period, producing a difference of 47% in its duration for the three years. The estimated difference of 55 days in the ice duration might be very relevant to shifting ecological relationships in terms of light availability, temperature and stratification. In temperate lakes of Canada, the ice-off dates in a 6 year series ranged 34 days (WYNNE ET AL. 1996). The ice cover duration in Lake Müggelsee (Germany) varied over 100 days in consecutive years (ADRIAN ET AL. 1999). This variation in the duration of ice cover has been suggested as a potential uncoupler for trophic elements (WINDER AND SCHINDLER 2004).

Lake Limnopolar shows a thermal pattern that falls between that observed for cold and temperate thereimictic lakes. This pattern seems to be characteristic for the lakes near this site. It has been reported for other lakes from the maritime Antarctic region, including Deception Island (DRAGO 1989), South Orkney Islands (HEYWOOD 1967) and King George Island (DRAGO 1980). This pattern is characterized by circulation only during summer season. Lakes following this pattern show a seasonal cycle similar to dimictic lakes, though without the summer stratification. On the other hand, an increase in conductivity as summer advances is noted as a general trend over the different years studied, being higher just after the ice thaw. This trend also agrees with that observed in other polar lakes (BORGHINI ET AL. 2008 and citations therein) and is likely a response to an enhancement in ion concentration produced by the increase in the drainage from the ice melting. BORGHINI and co-workers (2008) further state that, although interactions between the aquatic ecosystems and the surrounding lands in these sites are greatly restricted during winter, in ice-free areas such as Byers Peninsula, however, they increase during the thaw season, coinciding with the enhancement of biological activity.

As our results demonstrate, wind is also an important climatological mechanism associated with the air-water boundary and turbulence in the lake. Actually, it can be considered the major source of kinetic energy affecting lake. Shear produced by wind over the lake surface extends through the water column, causing water motion in excess of the normal flow originating from the lake inlets. In contrast, motions produced by thermal flux or density-driven currents dominate when the lake is ice covered. In this case, we found that the circulation pattern of water in the lake is critically modulated by the ice. This ice sheet largely controls the stability of the water column, since the wind effect is reduced and inverse water

stratification remains under the ice cap. For summers 2001-02 and 2002-03, convective mixing through the turbulent surface cooling is responsible for the thermal oscillations observed. The time-series analyses show that this atmospheric forcing is produced on a daily scale, though the more pronounced fluctuations are caused by episodic strong wind events. Thus, in this case, it is clear that the combination of relatively light winds and low solar radiation causes a negative net heat flux that leaves the lake as sensible heat.

The net heat flux in the lake can be approximated as the residual net radiation, lowered the sum of sensible and latent heat flux. The reliability of our results can be discussed at this point because we are not considering the latter term. However, latent fluxes out of the lake during summers 2001-02 and 2002-03 should be small since neither freezing nor thaw events took place during the periods studied. We further assume that the evaporative cooling, namely, a latent heat flux in the direction of the atmosphere, is insignificant, which is a reasonable argument for a cool environment as in this case. Hence, heat loss to the environment occurs mainly because water temperature exceeds ambient temperature, that is, in the form of sensible heat. During these ice-free periods it is evident that the cycle of energy storage in the lake follows that of solar radiation. In contrast, sensible heat fluxes are in the opposite phase, with minimum values occurring normally at noon and maximum values in the afternoon and night. Further, only wind bursts up to approximately 10 m s^{-1} induce a total mixing within the lake, deduced by the important heat losses produced during these events. Computing the wind velocities measured over the three summers together, as 30 min averages, only 12% of them are equal or above this magnitude (Fig. 4.35). In our opinion, this has some interesting implications. Although it is evident that lake is subject to continuous wind stress, it is possible that turbulent events high enough to produce mixing of the entire water column only occur occasionally during the summers. It led us to think that a certain isolation, greater than expected, may exist between the central basin and the surface layers of the lake.

Continuing with the idea exposed previously, a certain heat capacity of the lake is suggested by the delays observed in the warming and cooling periods in response to the atmospheric forces, which demonstrates that the later operate in lag periods. This suggests a certain thermal inertia despite the middle depth character of the lake. Further, this is in agreement with that deduced from the relative depth of the lake, with a value (3.24%) that implies moderate resistance to wind-induced mixing. It is also possible that the kettle morphology of the lake, which is not implicit in this parameter, provides some opposition to the total circulation of water.

Anyway, because our study holds only in the case of this particular lake, we can not directly attribute the influence of lake morphology in the regulation of energetic flux to other lakes of the region.

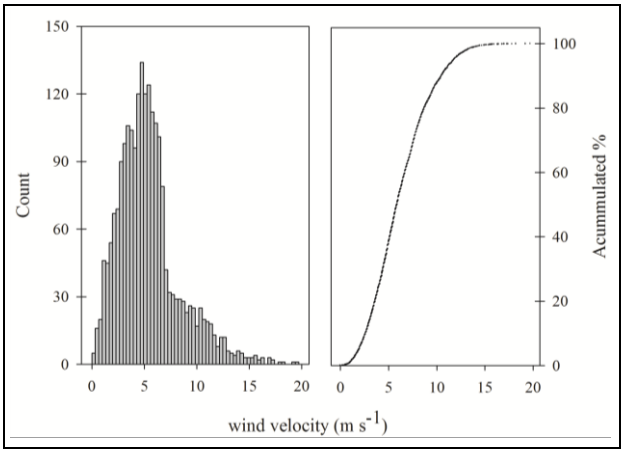

Figure 4.35. *Histogram and accumulative percentage that illustrate the distribution in the occurrence of different wind velocities in Byers Peninsula. Plots are constructed with date from three summers studied in this chapter. Results show how the occurrence of winds velocities strong enough to break thermal structure of water column (around 10 m* s^{-1}*) is restricted to around only 10% of time.*

In contrast to previous seasons, data obtained over summer 2003-04 allows for a better observation of the seasonal patterns in the thermodynamic and mixing processes for an Antarctic lake throughout the course of thaw period. As discussed previously, it is evident that the presence of ice cover during this summer prevented both evaporation and wind, which allows the lake to store the entering heat, resulting in a progressive water temperature increase. These results demonstrate the way by which the heat is trapped in the ice-covered lake. However, the results also suggest that different mechanisms govern depending on the particular period. For example, the heat gained in the brief period during the dam break likely is mainly generated by cells of warm water coming from the inlet. The increase in air temperature over January 6^{th}-7^{th} at summer 2003-04 could explain an incipient thawing. A remarkable increase in conductivity was found to parallel thawing, likely due to a higher concentration of ions dissolved in the inflow water. Nevertheless, it is possible that the turbulence associated with this process also produces an upward flux by removing the bottom sediment.

Later, from approximately January 24th to the beginning of February, the lake warmed likely from the increase in the incident solar radiation at the surface due to the snow and ice vanishing. It is known that heat flux from solar radiation gains relevance when it coincides with the thickness of snow cover (BENGTSSON AND SVENSSON 1996) and, as in our case, just before loss of the ice cover (BENGTSSON 1996). During these periods, the incoming radiation may warm water immediately under the ice, producing convective plumes that penetrate down into the water column beneath, as explained by VINCENT (1988). On the other hand, the highest heat flux out of the lake occurred during the ice thaw. This heat was released back into the atmosphere, from the ice thaw, probably mainly in the form of latent heat.

In addition to the external forces, the lake's features account for the energetic fluxes observed. At summer 2003-04 for example, in-lake characteristics primarily regulate the overall energy coming into the lake. Thus, it is the detailed analysis of the ice cap evolution which explains better the progress of lake warming rather than variations (both diurnal and seasonal) of the incident solar radiation. From December to later mid January the lake surface was covered with a uniform layer of snow that produced a notable albedo. During this period, a significant increase of heat in the lake occurs as a consequence of the input of warmer water from the inlet. This heat is efficiently stored in the lake by the absence of outgoing turbulent flux at the surface. After this period, a slight temperature increase begins to melt the snow and a higher occurrence of bare ice is observed. Finally, just after all the ice thawed, the lake surface was often comprised of ponded ice, difficult to step on. In the same manner of the dam breakdown, the variable weather conditions also appear to be the cause of ice-out acceleration. For example, enhanced air temperatures from January 20th-22nd coincided with sustained reduction in solar radiation. This occurred as a consequence of the generally cloudy conditions observed during this period (mid January – February), likely favoring a greenhouse effect, which explains the net warming despite reduced solar radiation. Likely, this effect accelerated the disappearance of the snow cover, the consequence of which is an important decrease in the lake surface reflectance. In addition, it is possible that water from precipitation and snow melting can pass through the ice cap fissures during this period.

MIRONOV ET AL. (2002), by examining the penetrative convections taking place in several ice-covered lakes, highlighted the importance of the dynamic effects caused by this type of convection when temperature is close to those of maximum water density, despite low salt concentration. In our case, the flux of thermal energy

from the surface into the deep layers is manifested by the increase observed for the vertical eddy diffusion (K_Z) during the quasi-steady state period for summer 2003-04, from middle to late January. However, discrepancies arise depending upon which diffusing substance or temperature is used in the calculations. These discrepancies arise because of potential differences between the heat and solute diffusivities. In any case, our observations agree with the regularly observed decrease in eddy diffusivity with depth, which display minimum values in or just below the thermocline (JELLISON AND MELACK 1993) or near the midpoint of the water column (ELLIS AND STEFAN 1996). Furthermore, our estimates fall within the range of those reported by LERMAN (1988) for stratified lakes (10^{-2}-10^{2} cm^2 s^{-1}). However, they are considerably higher to those observed in the permanently ice-covered Lake Fryxell in the Dry Valleys (AIKEN ET AL. 1991), which yielded values of 5.9×10^{-5} cm^2 s^{-1}. Besides AIKEN AND CO-WORKERS, other authors have also indicated that there is extremely little vertical mixing of this lake (SPIGEL AND PRISCU 1998, KEPNER ET AL. 1999). In our opinion, these data exemplify the notable contrast between the stability of perennially ice-covered lakes in the continental region and that observed in our case, even when Lake Limnopolar is covered by ice.

Following the conceptual explanation of WELANDER (1968), who considers vertical diffusivity a decreasing function of the Brunt-Väisäla frequency, it is possible to establish a relationship between this parameter and water column stability via the expression $K_Z = a(N^2)^b$. For several reasons this relationship is difficult to solve. First, in our case, N^2 originates from the CTD profiles, whereas K_Z is estimated from the continuous record of the thermisthors set at different depths of the lake. Therefore, although both agree in the description of the water column structure, they are constructed with different spatial and temporal resolutions, thus direct comparisons between them are difficult. Next, the thermal structure does not necessarily reflect density structure, particularly for the temperatures in Lake Limnopolar and if chemical gradients are involved. Hence, as noted MICHALSKI AND LEMMIN (1995), in the lower layers of lakes with a depth of <50 m, the temperature gradient becomes too small to obtain consistent results. This could be our case, in which an inverse thermal stratification implies slight temperature differences. In lake Limnopolar, both temperature and salinity gradients account for the onset of stratification, but each to a different extent. A particularity of the equation state of water is that it reaches its highest density at temperatures over the freezing point (3.94 ºC). This anomaly implies that water colder than this temperature becomes trapped by buoyancy above the warmer water. At temperatures close to this thermal anomaly, the contribution of the temperature gradient ($\delta\theta/\delta z$) to stability is low due to the small thermal expansion coefficient (RAVENS ET AL.

2000). In these cases, the salinity gradient ($\delta K_{20}/\delta z$) largely determines the N^2 profile. The higher salinity of the inflow water, though slight, is likely the major cause of the buoyancy flux responsible for the observed deep water strata.

Despite the observations previously stated, our estimations of eddy thermal diffusion coefficients in the water column fit into the general picture that we have of eddy transport occurring in stable water columns. Therefore, it is evident that the eddy diffusivity is controlled by the vertical density distribution. In our case, the eddy diffusion coefficient (K_Z) decrease in layers below pycnocline with a corresponding increase in Brünt-Väissäla frequency (N^2) values, which are higher just over the steep chemical gradient. Here, the higher stability of the water column that is generated by the salinity gradient acts as a barrier to diffusivity. This relationship causes the convective currents to reach only the deepest layers once stratification becomes weak. As observed in figure 4.29, over time this heating results in erosion of the upper portion of the picnoclyne. Formally, *Kz* can be assumed then as a function of depth variation and water density.

Returning to the idea that K_Z depends, to some degree, on N^2, there are additional observations that provide a context for our results. The higher N^2 values observed in Lake Limnopolar are on the order of $2x10^{-3}$ s^{-1} and the K_Z values are in the range of $6.31x10^{-6}$ - $5.87x10^{-5}$ cm^2 s^{-1} and $3.42x10^{-6}/3.87x10^{-5}$ cm^2 s^{-1} for the layers over and below pycnoclyne, respectively. Considering that, the estimate of coefficient *b* from the function $K_Z=a(N^2)^b$ should render values below -0.5. Presuming the limits established by WELANDER (1968) to determine causality for the turbulence phenomenon, this value of *b* indicates that, in our case, local shear and internal waves rather than large-scale processes generate mixing predominantly. It is important to mention, however, that under this reasoning, we apply a value for coefficient *a* of $0.04x10^{-5}$, which has been obtained for an ice covered lake (ELLIS ET AL. 1991).

The reliability of our K_Z estimates at summer 2003-04 might not be the same depending on the underlying temporal and/or spatial occurrence. In our case, we note a higher variance of the temporal derivatives in the uppermost layer of lake (slice 1-2 m). Some authors, who have also used the flux gradient method to estimate K_Z, indicate a higher variance in the integrals of the surface layers of the water column, indicating that this phenomenum is caused from greater exposure to surface effects (STAUFFER 1992, MICHALSKI AND LEMMIN 1995). However, they also refer to the stress caused by wind, which should be excluded in our case due to the presence of a thick ice cap. However, there are non-conductive processes such as

radiative heat transfer, which affects surface layers and may also introduce uncertainty.

The mathematical formulation that we have used is based on Fick's law of diffusion. This calculation presumes that heat is always transported by diffusion and not by advection, which is not entirely true. For example, horizontal heat flux could introduce bias in our estimations. The water influx in the lake during mid January might, for instance, lead to an overestimate of the K_Z values below the pycnocline. It is also possible that heat from the borders of the ice causes advection towards the bottom, as observed in other lakes (BENGTSSON ET AL. 1996). Likely, the meltwater from the surrounding catchment reaches the lake through cracks in the shoreline, where the ice meets the shore. Another factor not included as a source/sink term in our temperature model, however, is the role of heat gained from the bottom sediment. It can be an important factor, mainly in ice-covered lakes (FANG AND STEFAN 1996). Assuming these limitations, we then consider the values of eddy diffusivity observed just above the pycnocline during the period after the dam breakdown and before the ice thaw as the most realistic. These values are more realistic because they occur under relatively calm conditions, in contrast to other periods during which unsteady turbulence seems to prevail.

The influence of the physical conditions on nutrient dynamics was also observed in the present study. The impact of lake turnover might explain the increase in the nutrient concentrations observed at summer 2003-04 after the thaw and coinciding with a major convective regime in the lake; this also includes input from the catchment. Over summer 2003-04, the development of convective currents when stability weakened likely led to an increase in particulate nutrients that peaked just when the ice thaw began. Nevertheless, higher temporal and spatial resolution would be necessary to obtain more conclusive results. In contrast, a limited exchange of nutrients is supposed to occur across the chemocline when stability is higher. During this period it is possible that chemical fluxes take place in the opposite direction (i.e., downward), producing a diapycnal nutrient diffusion. This phenomenon is based on the idea that some nutrients may be released from the ice when it thaws. In fact, an increase in nutrient concentration at surface layers has been observed as a consequence of debris-containing ice melting in other Antarctic sites (ROBERTS ET AL. 2004). These nutrients accumulate in the ice from the exposed soils within the catchment caught in the wind or by the aerosols load (PRENDEZ ET AL. 2009). This accumulation could be even stronger for Lake Limnopolar given the snow covering the ice cap, which contains significant nutrient content originating from atmospheric precipitation, as suggested by our analyses (see table 4.3).

We hypothesize that the pelagic release of DOC might play a secondary role in fueling bacterial production for many of the lakes in Byers, including Lake Limnopolar. This is supported by the generally low Chl-*a* concentration observed (see chapter 3). Thus, other sources might account for heterotrophic production, such as the allochthonous DOC produced in the watershed or the in-lake photosynthetic benthic production (CAMACHO 2006a). Both DOC origins are very sensitive to variations in ice thawing, the former because of the reduced production at freezing temperatures or when the catchment is covered by snow, and the latter because of the low irradiance availability when lake is ice covered. As commented in the previous chapter, this allochthonous supply has been suggested in other Antarctic locations, as in the McMurdo Dry Valleys, where the water circulating near the catchment maintains a 10-fold higher DOC concentration over those originating directly from glacier ice melting (LYONS ET AL. 2007). Additionally, as observed by AIKEN ET AL. (1999), in Lake Fryxell a diffusion of fulvic acids may also result from the sediments, which could be even higher in the case of Lake Limnopolar, considering its lower stability.

There are some trends of heterotrophic metabolism in the lake that can be inferred from the CDOM analysis. According to MCKNIGHT ET AL. (2001), variations in the position of peaks in the EEM matrices can be interpreted as a relative increase of the aromaticity of the fulvic acid components. These authors established the 450 nm/500 nm emission ratio from 370 nm excitation as an appropriate measure for the microbially-derived fulvic acids (ratio approximately 1.9) or terrestrially-derived fulvic acids (ratio approximately 1.3). Since our analyses were not calibrated with fulvic acids as recommended MCKNIGHT and co-workers to standardize the influence of differences in fluorimeter configuration, it is inappropriate to directly compare our results with these index values. Nevertheless, the peak displacement in the C emission toward lower wave-lengths that occurs in our samples on January 30th indicates likely a greater occurrence of microbially-derived DOC with respect to its precursors. The spectrum that we obtained closely resembles those of isolated fulvic acids obtained from Lake Fryxell and Pony Lake, which are compiled in the work of MCKNIGHT and co-workers (2001). Previous observations from Lake Fryxell, however, suggest a low biological influence on the DOC profiles. Probably the major processes controlling DOC in that lake are inflow and diffusion from tributaries and bottom sediment, respectively (AIKEN ET AL. 1996). Otherwise, exudates from Arctic microbial mats (Ward Hunt Ice Shelf , 83°020N, 74°000W) have shown ratios in CDOM matrixes that demonstrate a composition based on complex molecules or allochthonous precursors (MUELLER AND VINCENT 2006). These same authors argue for existence of certain water-

soluble compounds, such as extracellular polymeric substances (EPS) and oligosaccharide mycosporine-like amino acids, which can easily be released into the overlying water. However, concerning the latter, the recurrent lack of protein-like peaks in our CDOM matrixes leads us to think that this input has maybe low significance.

Summarizing, we have shown how physical trends in the lake are affected by environmental forces which, in turn, largely depend of the of ice cover condition. Our study demonstrates how, during three consecutive years in which the meteorological conditions were quite different, ice dynamics are especially sensitive and very much subject to variations. In the present chapter, we have explored periods that differed dramatically in the ice condition of lake. However, we hypothesize that smaller variations in some factors, such as the amount of snow cover over the ice and the ice characteristics, may significantly alter the pattern of inverse stratification, which only occurs when the lake is ice-covered. In our opinion, further attention has to be paid thus to the potential disagreement between changes observed locally and those predicted by forecast models due to these year-to-year variations. To project more precisely the long-term trends in lake ecosystems, studies would, consequently, have to be large enough to integrate the inter-annual variability that we have highlighted here.

5. Plankton dynamics in Lake Limnopolar in three meteorologically contrasting summers

5.1. Introduction

The physical processes that take place in lakes can potentially alter the dynamic of aquatic organisms. The biological production in Antarctic ecosystems is constrained by the imposition of one or several environmental extremes. Among them is the timing of the ice cover. It affects both internal and external processes running the flow of energy into the system. For instance, the availability of nutrients, the thermal structure of water column, or the light regime, are all largely influenced by the characteristics and durability of the ice cap. Indeed, the length of the lake's ice-free period, combined with other parameters, has been showed to affect life-cycles and patterns of geographical distribution of biota (SCHINDLER 1997), as well primary production (ADRIAN ET AL. 1999; PARK ET AL. 2004), thus affecting the trophic interactions and biomass transfer occurring in the system (CAMACHO 2006a).

In lakes, an earlier and larger ice-free period implies both the increase of light availability and nutrient supply. Numerical models predict that global warming will increase the air temperature by 1.5 to 6 ºC over the next 100 year (IPCC 2001). Even taking into account the more conservatives forecasts, it would translate in large changes on freezing and thawing dynamics in the Antarctic maritime region as typical summer temperatures in this region are slightly above the melting point of water. Indeed, an earlier opening has been observed in lakes from Signy Island in recent years (QUAYLE ET AL. 2002). Compared to lakes from Signy Island, the ice-out in Byers takes place several weeks later due to the insulation provided by the higher snow precipitation (JONES ET AL. 1993), which confers to Byers a particularity on its regional context.

The pelagic production in perennial ice-covered lakes relies mainly on the internal carbon budget (DORE AND PRISCU 2001). It implies that the inter-annual variability of plankton dynamics in these lakes responds overall to overwintering strategies and/or, if they are present, the trophic interactions between organisms (MCKNIGHT ET AL. 2000). This situation is completely different in the maritime region since the warmer climate allows the ice-melting at summer. The total or partial disappearance of the ice allows lake's productivity to be significantly subsidized by allocthonous inputs of nutrients (PRISCU ET AL. 1998). Under these circumstances, important changes of species composition may occur (ELLIS-EVANS 1996a). By contrast, lakes from continental region seem to lack patterns of species succession during summer (SPAULDING ET AL. 1994; LIZOTE ET AL. 1996). Aside from the maritime region, summer dynamics have been observed merely in the

perennially ice-covered Ace Lake in the Vestfold Hills, but it responded to a significant removal of snow from the lake surface (BURCH 1988).

In the previous chapter, we showed how ice dynamics in Lake Limnopolar dictate the thermal regime and therefore the stability of the water column. The coupling between phytoplankton dynamics and the thermal structure of water column has been described both in Antarctic (PRIDDLE ET AL. 1986, IZAGUIRRE ET AL. 1993) and temperate lakes (KELLEY 1997, ADRIAN ET AL. 1999, PARK ET AL. 2004). These dynamics are often explained by changes in the water column stability. When turbulence is low, such as occurs in ice-cover lakes, only those organisms with reduced sinking rates remain in the water column. On the contrary, a higher turbulence facilitates large and non-motile species to dwell in the pelagic compartment. Other example of how the physical structure of water column may affect the primary production is, for example, by increasing the nutrient supply in the euphotic zone. Besides, the ice melting augments the water flux into the lakes and consequently the supply of nutrients via runoff. As pointed out in the previous chapter, internal high-frequency waves occur in Lake Limnopolar, which can be associated with this increase of nutrient fluxes (MACINTYRE 1999). In addition, an earlier thawing may enhance the availability of phosphorus in the water column via the sediment removal (BLENCKNER 2005). Some of these processes were described in Lake Fryxell (MCKENNA ET AL. 2005), a lake located at the lower end of Taylor Valley in Victoria Land. There, it was observed how some plankton reach their maxim densities coinciding with the maximum light availability and just when the ice melting allows to tributary water comes into the lake.

A coupling between adjacent trophic levels is required to efficiently exploit pelagic resources. This trophic coupling favours elevated re-mineralization rates of nutrients in the pelagic compartment by minimizing the losses generated by sedimentation (STONE AND WEISBURD 1992). On the other hand, the availability and quality of food also have profound implications in the growth of copepods (TWOMBLY ET AL. 1998), which dominate in Lake Limnopolar. It has been observed for instance that the trophic status regulates the life cycle of *Boeckella poppei* in lakes from the Antarctic Peninsula (IZAGUIRRE ET AL. 2003), in such a way that this copepod display either an univoltine (i.e., a single reproductive event during a year) or a multivoltine life cycle (i.e., several reproductive events during a year) in oligotrophic or meso-eutrophic lakes repectively.

We need to identify how physical processes occurring within the lake, but also those occurring in the surrounding catchment, regulate the food web dynamics. As shown in the previous chapter, environmental conditions in Lake Limnopolar

during summer 2003-04 differed from preceding years. This summer was characterized by a delay of 55 and 25 days in the ice-out timing in comparision to 2001-02 and 2002-03 respectively, and also a much short free ice period. We carried out a study of summer plankton dynamics in the lake during these consecutive expeditions. Based on the results obtained, both the spatial and temporal distribution of pelagic organisms are discussed. The structural role of different environmental factors such as the thermal regime, and the availability of light and nutrients is then reasoned.

Energy fluxes along the trophic food webs can be envisioned by observing the size distribution of the organisms (GAEDKE 1993, GAEDKE ET AL.. 2004). In the present chapter, we address an analysis of the body size distribution of plankton community in Lake Limnopolar during summer of 2003-04. Measurements spanned the period from final December 2003 to mid February 2004, thus embracing the end of the ice-cover period and the thawing. Biomasses of microbial components and metazooplankton were seasonally determined. Temporal trends of pelagic body size spectra were then outlined using a Pareto power function to obtain information on functional aspects. It is a size-based analysis that explores aspects related with the structure and function of ecosystems (COHEN ET AL. 2003), in particular those related with the efficiency of carbon transfer through the food web. Our study is guided to predict the likely outcomes of future climatic warming on the productivity and functioning of lakes from this region, given that ice dynamics can be strongly influenced by climatic variation (LATIFOVIC AND POULIOT 2007).

5.2. Methodology

5.2.1. Sampling procedures

A biological sampling was conducted in parallel to the physical-chemical survey carried out in Lake Limnopolar during summers 2001-02, 2002-03 and 2003-04 (see previous chapter). The sampling of photosynthetic pigments and pelagic organisms (i.e., virus-like particles, bacteria, autotrophic picoplankton, nanoflagellates, phytoplankton, ciliates and metazoan) was performed following procedures described in chapter 3. Samples for virus-like particles (VLP) counts were only obtained during summer 2003-04. Samples at summers 2001-02 and 2002-03 were collected at 2 meters depth, whereas at summer 2003-04 samples were obtained from two depths (1 and 4.5 meters below ice). The temporal resolution of sampling

also differed between summers. At summer 2001-02 the lake was sampled three times, two times at summer 2002-03 (for some parameters) and six times at summer 2003-04. The possible bias produced by the vertical migrations of organisms was standardized by sampling approximately at the same time at all dates (noon).

5.2.2. Biomass calculations and analysis of plankton community size structure during summer 2003-04

To describe trends in the pelagic food web structure, a sizing and biomass estimation of plankton members was performed at summer 2003-04. For biomass calculations, we proceeded as described in section 2.3. The biomass size spectra obtained at different dates were then analyzed by means of the probabilistic Pareto power function (PARETO 1897):

$$F(x) = 1 - \left(\frac{k}{x}\right)^{c} \qquad \text{(Equation 5.1)}$$

The underlying parameters of the equation (c and k) define the shape and scale respectively. As indicates VIDONDO ET AL. (1997), if data are plotted on a double-logarithmic scale, c and k can be then approximated respectively as the slope and the antilogarithm of the ratio intercept/slope of the linear function. Following this procedure, the subsequent cumulative distribution of probability can be obtained:

$$\log \Pr(S > s) = c \cdot (\log k - \log s) \qquad \text{(Equation 5.2)}$$

where s is the size (body length in our case) of an individual taken at random and S is a threshold size expressed as a function of s. For each organism, the term $\Pr(S>s)$ is obtained as the fraction of all individuals larger than or equal to itself. In our case, the body length of each organism was standardized by dividing it by the minimum body length observed in the sample. This allows to better compare the obtained results among different samples.

The obtained curve of body size distribution yields a slope of -1 when highest evenness occurs; consequently, those values deviated from this indicate that biomass distribution in the food web is unbalanced. This means that spectra dominated by small organisms will display higher values of c, whereas lower values should indicate a higher relative abundance of large organisms. We computed the

size spectra of both depths separately in order to explore the occurrence of vertical heterogeneities. A trapezoidal integration of the water column seems, at any rate, inadequate because of the coarse resolution of our sampling (two depths). With the data obtained for each depth, a regression analysis was made employing a least squares linear regression.

5.3. Results

5.3.1. Virus-like particles and bacterioplankton abundances

The abundances of bacterioplankton (=heterotrophic picoplankton; HPP) varied in a similar range during the different summers, although they were dependent on the ice condition in lake (Fig. 5.1). During the summer 2001-02, highest abundances up to $2x10^6$ cells mL^{-1} were observed when ice was melting out. Afterwards, at ice-free conditions, bacterial densities fell to levels between $1.3\text{-}1.5x10^6$ cells mL^{-1}, which nearly coincided with numbers observed at 2002-03 during the same period. In summer 2003-04 densities evolved differently. Thus, they showed a progressive increase from 0.8 to $1.6x10^6$ cel mL^{-1} that peaked when lake was totally free of ice. These values were akin to densities observed during 2001-02 under these same circumstances. On the other hand, bacterial abundances at the bottom layers during summer 2003-04 varied in a higher range ($16.02\text{-}22.43\ x10^6$ cel mL^{-1}). The latter showed also an increasing tendency along time, except at late January when they declined slightly.

The abundances of free virus-like particles (VLP) in the water column were also determined at summer 2003-04 (Fig 5.2). Similarly to bacteria, they were not uniformly distributed in the water column and displayed also notable changes with time (Fig. 5.3a). The counts showed concentrations ranging from $3.94\cdot10^6$ to $5.51\cdot10^6$ VLP mL^{-1} at surface and $6.93\cdot10^6$ to $13.7\cdot10^6$ VLP mL^{-1} at deep. This resulted in viruses/bacteria ratios (VBR; Fig. 5.3b) that fluctuated greatly in the deep layer, varying from 3.47 to 7.29, with high values occurring at middle of January. By contrast, lower and more homogeneous ratios were observed in surface (2.88-5.36). In any case, the lowest ratios coincided at two depths at February, just when total mixing of water column occurred.

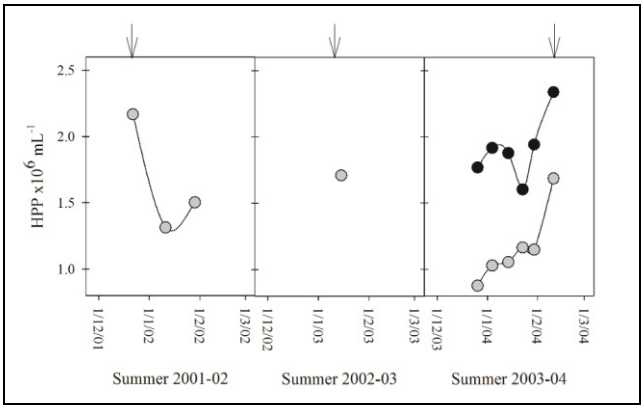

Figure 5.1. *Abundances of bacterial population (HPP) in Lake Limnopolar during the three summer seasons studied. Samples are from mid-surface (grey) and bottom (black). Arrows indicate the first date at which lake was observed to be ice free.*

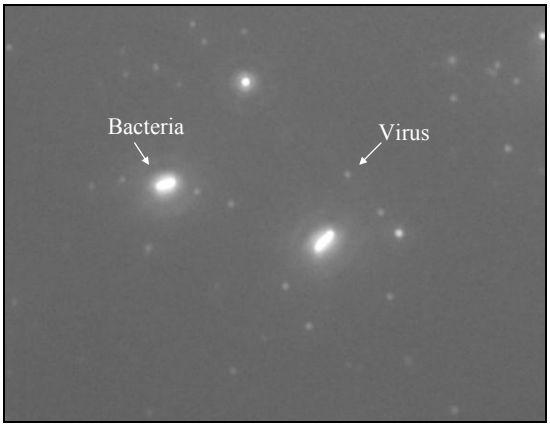

Figure 5.2. *Epifluorescence microphotograph showing bacteria and viruses labelled whith the SYBR-Green probe*

5.3.2. Photosynthetic pigments concentrations

The total algal biomass at summer 2003-04, measured as the concentrations of chlorophyll-*a* (Chl-*a*) (Fig. 5.4), was low and ranged 0.03-0.31 µg L^{-1} at surface and 0.08-0.18 µg L^{-1} at deep layer respectively. When the survey started at this summer (late December), concentrations were in the lower values of the range, being higher at deep. They remained approximately constant until mid January. After that, a peak occurred at surface at 23rd Jan, although maxim concentrations at deep were observed a week later. In 2001-02 and 2002-03 the algal bloom under the ice was

not evidenced because the samples were collected after the ice-melt. At these summers, Chl-*a* concentrations measured were in the narrow range of 0.13-0.19 μg L^{-1}.

The changes in the weight ratios between the taxon-specific carotenoids and Chl-*a* are shown in figure 5.4. Fucoxanthin/Chl-*a* ratio showed a constant increase along time, reaching relative concentrations of 0.23 and 0.1 at summers 2001-02 and 2003-04 respectively. These relative amounts were in the range of that observed in February of 2003, when the lake was also totally ice free. In contrast to 2001-02, in summer 2003-04 the fucoxanthin/Chl-*a* ratio mimicked Chl-a trends indicating that the increase in algal biomass was mainly due to the growth of the algal groups identified by this pigment (chrysophytes and/or diatoms). Changes in lutein ratios in summer 2001-02 were similar to those observed for fucoxanthin. Thus, the lutein/Chl-*a* ratio showed a 4-fold increase when lake was ice free in relation to that measured at the first sampling date, just when the lake surface was still partially frozen. However, no significant increase (between <0.001 to 0.042) was observed for the lutein/Chl-*a* ratio in 2003/04.

The temporal trends of other pigments were additionally determined at summer 2003-04. For instance, the relative concentrations of chlorophyll-*b* (Chl-*b*) were somewhat higher at deep layer until mid-January, when higher amounts occurred at surface coinciding with the Chl-*a* maximum. More notable increases were observed for zeaxanthin, which peaked in the two depths also at mid-January. On the contrary, the relative amounts of violaxanthin were consistently lower in comparison to the former and not showed neither significant variation despite of the increases of Chl-*a*.

5.3.3. Phytoplankton community

The microscopic examination of samples evidenced a low grade of diversity within the phytoplankton community. Pictures of representative taxa are shown in figures 5.5(1), 5.5(2) and 5.5(3). Community was dominated by pico- and nanophytoplanktonic size fractions. Concerning diatoms, the group was represented totally by pennate forms, being mainly composed by the genera *Navicula*, *Nitzschia*, *Achnanthes* and *Fragilaria*. The pigmented chrysophytes subset was composed principally by two species of genera *Pseudokephyrion* and *Chrysococcus* cf. *rufescens*. Regarding to chlorophytes, the most frequently observed forms were non-flagellated genera *Ankistrodesmus antarcticus* and *Ankyra* sp. Other chlorophytes regularly observed were the pico-sized *Kirchneriella* sp. and the flagellate

Chlamydomonas sp., Cryptophytes were largely represented by *Chroomonas acuta* and, in a lesser extent, by other non-identified small forms (Fig 5.5(1)e). On the other hand, a part of the assemblage originated from unidentified small plastidic flagellates (5-6 µm) classified tentatively as chrysophytes corresponding to genera *Ochromonas* and *Chromulina*, and picoprasinophytes-like forms.

Pennate diatoms dominated the phytoplankton assemblage at summer 2001-02, showing densities that ranged 136-689 cell mL^{-1}, being higher just after the ice melting. Abundances of autotrophic picoplankton (APP) during this summer were always below 150 cel mL^{-1}. This notably contrasted with the higher abundances observed at summer 2003-04. Thus, in 2003-04 they averaged in surface $0.3x10^3$ cells mL^{-1} ± 0.2 SD, maintaining even higher numbers at deep layers, where they ranged from $7.7x10^3$ cells mL^{-1} to $24x10^3$ cells mL^{-1}. At summer 2003-04, chlorophytes showed a nearly homogeneous distribution with depth. They peaked at the early days of January, when densities were around 30 cell mL^{-1}. During this summer, the highest densities of cryptophytes took place somewhat later at mid-January, being both 14 and 12 cell mL^{-1} in surface and deep respectively. They were almost absent in the assemblage at the final of period, though a little population maintained at deep layers.

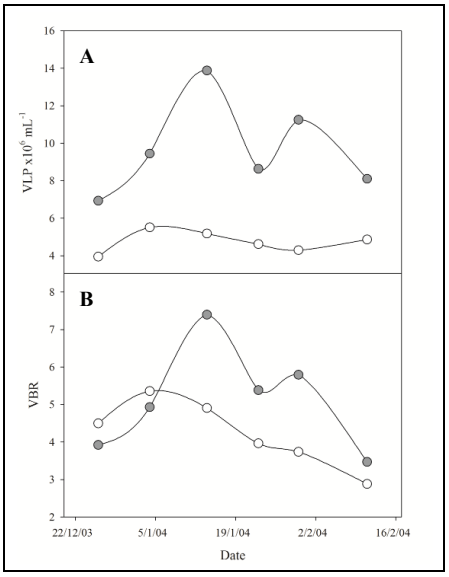

Figure 5.3. *Dynamics of virus like-particles (VLP) in Lake Limnoplar during summer 2003-04 at surface (white circles) and deep layers (grey circles). A) Abundances. B) VLP/bacteria ratios (VBR).*

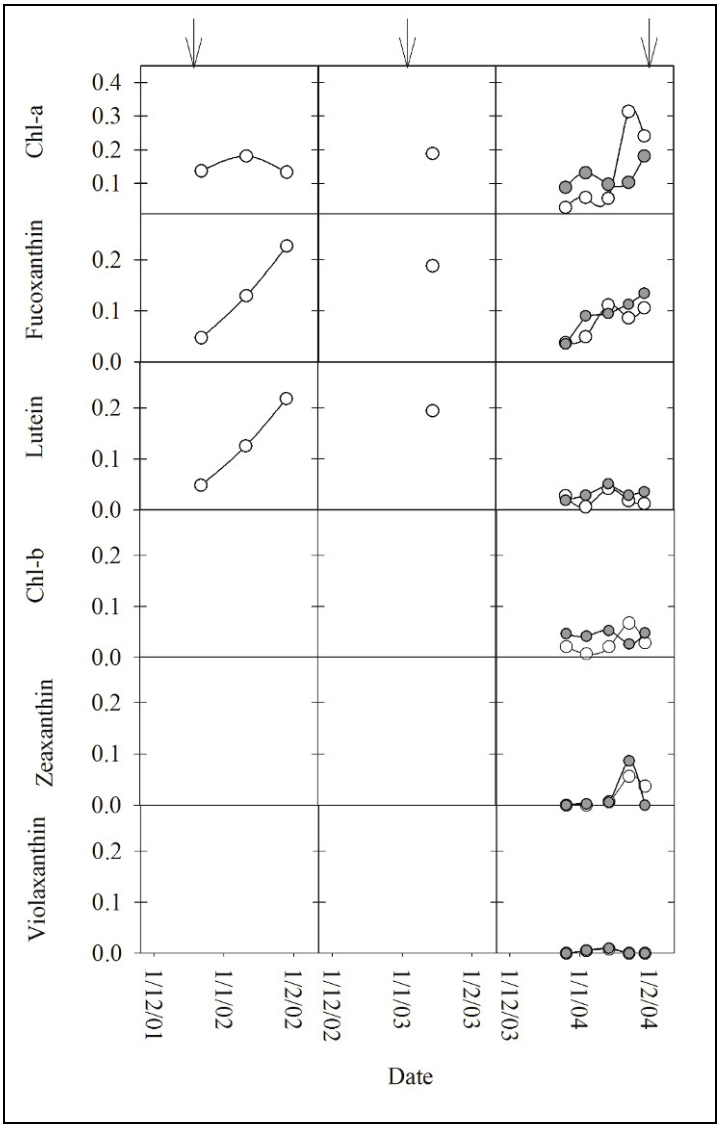

Figure 5.4. *Changes in pigments concentrations during different summers seasons at surface (white circles) and deep (black circles) layers. Concentrations of Chlorophyll-a are expressed as µg L^{-1}, whereas the rest are expressed as µg µg Chl-a^{-1}. Samples from 11st Feb at summer 2004 lack because they were lost during the transport to Spain.*

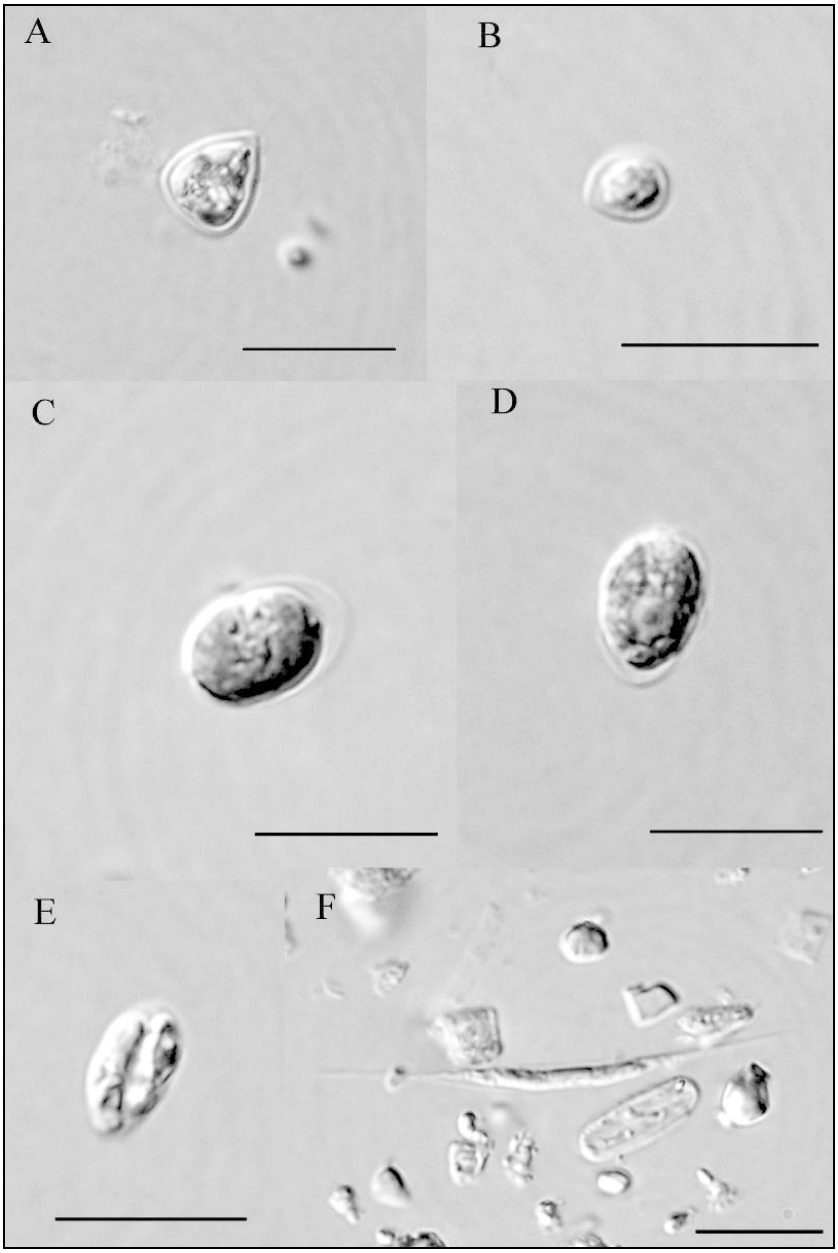

Figure 5.5(1). *Micro-photographies of major phytoplankton organisms observed in Lake Limnopolar at summer 2003-04. (A-D) Chrysoccocus spp. E) Cryptomonadal and F) Ankistrodesmus antarcticus. Bars indicates 10 μm in all cases.*

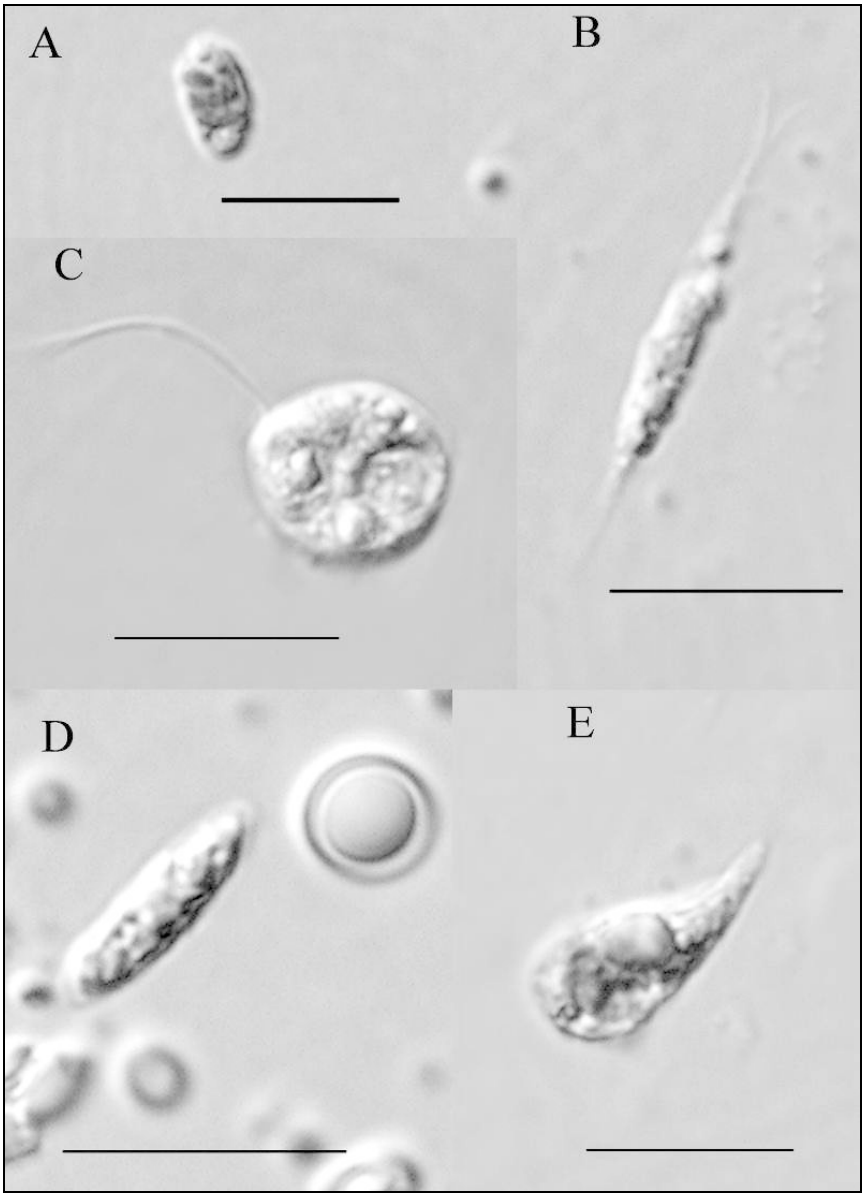

Figure 5.5(2). *Micro-photographies of major phytoplankton organisms observed in Lake Limnopolar at summer 2003-04.A) Chilomonas sp., B) Ankyra sp. C) Clamydomonas sp. D)Chilomonas sp. D) Chroomonas acuta. Bars indicates 10 µm in all cases.*

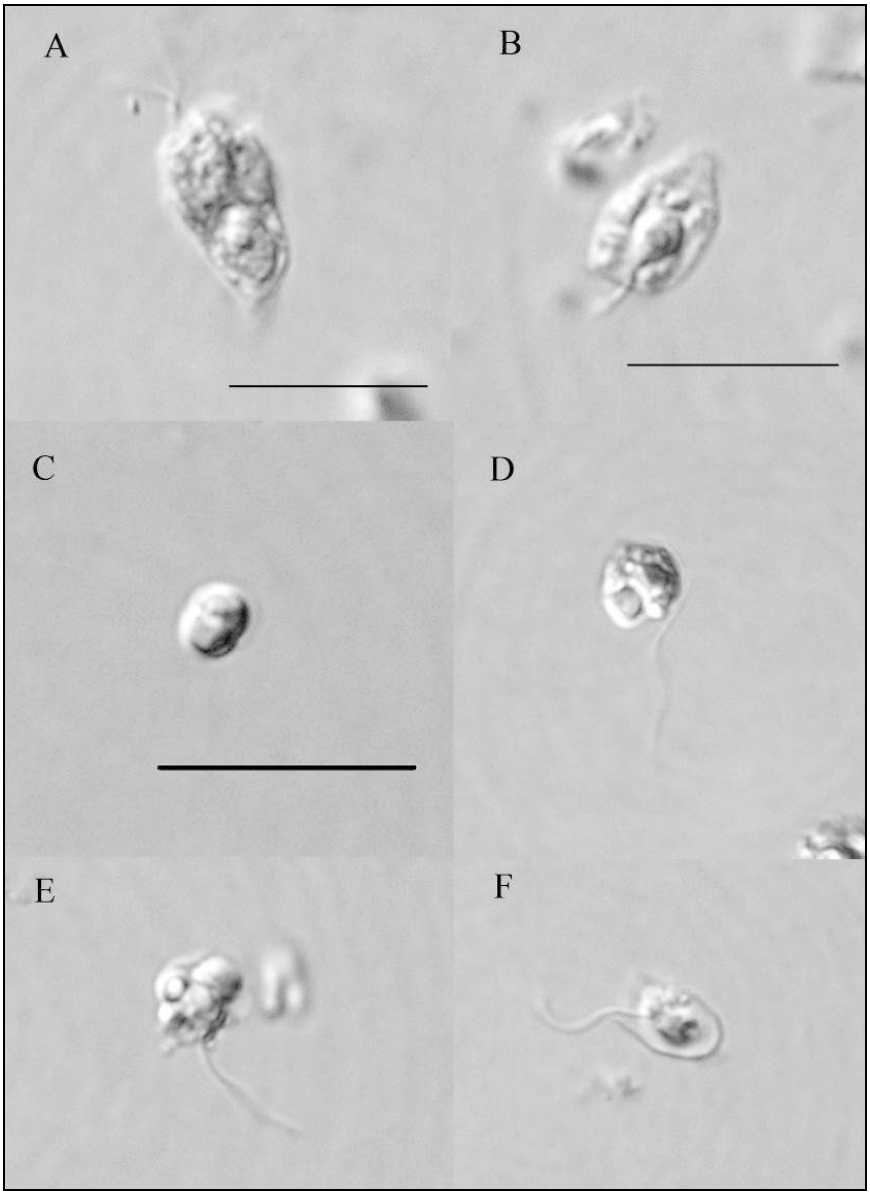

Figure 5.5(3). *Micro-photographies of major phytoplankton organisms observed in Lake Limnopolar at summer 2003-04.A) Cryptomonadal, B) uncapsulated chrysophyte, C)Picoeukaryont D) unclassificated nanoflagellate E) unclassificated nanoflagellate F)Pseudokephyrion sp. Bars indicates 10 µm in all cases.*

5.3.4. Protozooplankton

With regards to nanoflagellated protist, cell forms of 3-5 µm long dominated (Fig. 5.6), they were assigned to chrysomonads (similar to *Spumella* and *Oikomonas)*. The total numbers in surface waters varied similarly in summers 2001-02 and 2003-04, ranging from 115 to 526 cel mL^{-1} and 131 to 457 cel mL^{-1} respectively, with higher numbers occurring respectively during ice free and melting phases. In 2002-03, the abundance was below 200 cell mL^{-1}. At summer 2003-04, however, the added sampling of the deep layer revealed there a more pronounced variation. Hence, abundances remained below 50 cells mL^{-1} in both depths until 23rd Jan, when they augmented around ten folds at deep. An enhancement of the population was also observed in surface at this date although numbers were quite lower (174 cells mL^{-1}).

Ciliates diversity in Lake Limnopolar was particularly low, being composed during the three years by few nanoplanktivorous species of prostomatids among which *Balanion planctonicum* was the dominant specie. Ciliates population increased just after the ice melt out (Fig. 5.6), showing their higher abundances after the ice retreat. In summer 2001-02, ciliates reached and maintained highest densities between $2.2x10^3$ and $2.8x10^3$ ind L^{-1}. Densities observed at 15th February 2003, when lake was in similar conditions, were also in this range. In contrast, abundances in 2003-04 ranged in low levels between $0.2x10^3$ to $1.2x10^3$ ciliates L^{-1}, with the lower numbers occurring when lake was still covered by ice. As a general trend, they kept a relatively homogeneous vertical distribution, except at 23rd Jan, when numbers peaked at surface.

5.3.5. Metazooplankton

The metazooplankton assemblages during the three studied summers were composed almost only of the copepod *Boeckella poppei*. Some individuals of the Cladocera *Macrothrix oviformis* (after Kotov 2007) were also recorded, although they were nekto-benthic associated visibly to the bottom. Rotifers, which were largely represented by specimens of genera *Notholca*, were also present in samples but they were rare and numbers never exceeded densities of 0.3 ind L^{-1}. Otherwise, the anostracean *Branchinecta gaini* only was observed occasionally from the deeper or column-integrated net samples. Both anostracean and cladocers numbers were unclear. It is because they resided in the deepest layer so we suppose that they not were effectively captured with our sampling methods.

Concerning copepods, both the densities in surface waters (Fig. 5.6) and the evolution of population age structure differed between summers 2001-02 and 2003-04. During 2001-02, numbers recorded at surface were always below 1 ind L^{-1}, whereas at 2003-04 abundances varied in a somewhat higher range between 1-5 ind L^{-1}. A more precise data about the copepod distribution obtained during surveys at summer 2003-04 demonstrated, however, that this species migrates up and down in the water column, and at the bottom the density can be as high as 10-fold higher than at the surface. The vertical distribution during this summer was then particularly characterized by a retreat of individuals at deep layer during the illuminated periods, with total abundance varying there from 3 to 60 ind L^{-1}.

More feasible resulted to compare the cohort structure between the different summers. In the summer 2001-02, age structure of population changed progressively from a higher prevalence of undeveloped forms in last December to a cohort dominated largely by adults at the end of January. Here, the highest percentages of nauplii (4%) and copepoids (59%) occurred at the end of December. Subsequently, nauplii decreased and finally disappeared at the end of the studied period. By contrast, the distribution of copepoids and adults remained nearly constant until mid January, thereafter adults increased notably (89%). The population structure observed in summer 2003 at the same period and with the lake also free of ice differed little from the previous year. At that time, immature forms somewhat dominated, although neither nauplii stages were observed.

The more noticeable difference at summer 2003-04 compared to previous years relied in the fact that adults never dominated in the assemblage. Their relative abundances ranged 5-42%, whereas copepoids were always between 56-91%. Also in opposition to the previous years, two cohorts apparently occurred during summer 2003-04, seeing that two copepoids maxima took place at the beginning of January and at middle of February respectively. At this summer, the transition from nauplii and the early copepoids to the more developed stages occurred steadily. As a result, a great share of copepoids was noted from the begining to mid January, and subsequently a notable increase of adults occurred at late January. At the final of December 2003, we verify by using migration traps that part of the population migrated near the surface at night (Fig. 5.7). However, only a portion of the population participated, being this mainly performed by immature stages.

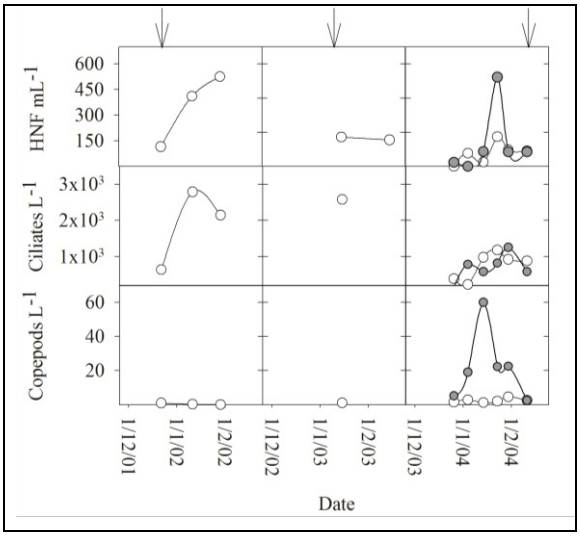

Figure 5.6. *Temporal evolution of grazers at surface (white circles) and deep (black circles) layers during different summers studied in Lake Limnopolar.*

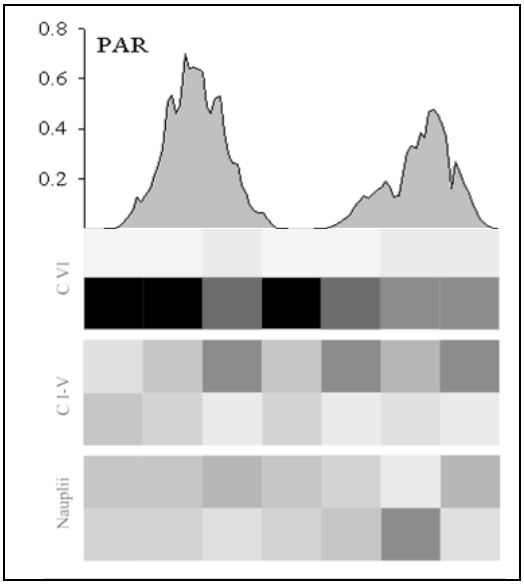

Figure 5.7. *A dimensional grey-scale plot showing the migratory behavior of different copepods stages in Lake Limnopolar at the final of December 2003. Black squares indicate the higher relative densities. The two lines of squares are surface and deep layers respectively.*

5.3.6. Carbon dynamics in plankton community at summer 2003-04

The bacterioplankton biomass in surface waters showed a gradual increase with time from 12.27 µg C L^{-1} to 23.58 µg C L^{-1}. At deep layers, phytoplankton biomass varied narrow around 5 and 10 µg C mL^{-1} until 30rd Jan, after that phytoplankton increased notably. Thus, the phytoplankton carbon peaked at both depths at same date (30th Jan), reaching values of 24.4 and 22.3 µg C L^{-1} at surface and deep respectively (Fig. 5.8), which basically coincided with trends observed for the concentrations of Chl-*a*. Later, when the complete ice-melting occurred (11st Feb), phytoplankton total biomass fell below 5 and 10 µg C L^{-1} in surface and deep respectively, which were similar levels to those observed at the beginning of this season. In terms of biomass, diatoms and chrysophytes constituted the higher proportions, particularly at surface, and largely determined the pattern of phytoplankton succession. Thus, when whole phytoplankton peaked, both groups comprised respectively up to 45% and 42% at surface and 39% and 27% at deep of total carbon. It contrasted with the observed for the autotrophic picoplankton (APP), which was only higher in terms of biomass from 14th to 23rd January at the deep layers, when entailed around 50% of total carbon.

The biomass of chlorophytes peaked on 6.1 and 5.0 µg C L^{-1} in surface and deep respectively at the early summer. In posterior dates, their relative dominance was lower than 4%. The biomass of cryptophytes was always low and never exceeded 4 % of total carbon, even when the lake was still ice-coreved. Otherwise, the biomasses of the unidentified small flagellates similar to *Ochromonas* and *Chromunlina* were nearly invariable over the summer at two depths. They were, in any case, somewhat higher at the beginning of summer, showing relative contribution to total carbon of 5% (surface) and 12% (deep). Other algal groups such as filamentous desmids and cyanobacteria were of minor importance and reached up to 1% of total biomass when peaked.

The seasonal evolution of the zooplankters' biomass is shown in figure 5.9. The biomass of ciliates ranged 0.1-0.85 and 0.1-0.53 µg C L^{-1} in surface and deep respectively, being higher just before the ice melting. The standing stock of the heterotrophic nanoflagellates (HNF) were similar and peaked also at this period, matching 0.32 and 0.96 µg C L^{-1} in surface and deep respectively. Following, the populations declined considerably at two depths. If data are computed jointly, the total biomass of protozoan at surface ranged from 0.16 to 1.17 µg C L^{-1}, while at deep they varied from 0.14 to 1.29 µg C L^{-1}. On the other hand, ranges of metazoan biomass were 0.77-17.6 and 12.5-313 µg C L^{-1} in surface and deep layers respectively.

As observed in the figure 5.9, protozoan and metazoan evolved differently. The protozoan biomass was higher at 23rd Jan, whereas metazoan peaked around one week early. The plots compiled in the figure 5.10 show variations along time of the carbon pool ratios between different trophic levels. The proportions of picoplankters in relation to protozooplankters were noticeably elevated (Fig. 5.10b), mainly as a consequence of the important pool of bacterial carbon. These ratios averaged in surface and deep layers 42.5±23.9SD and 82.1±58.6SD respectively. Ratios were higher when the survey started, especially in deep layers, subsequently decreased progressively until their minimum values (13.9-20.8) were reached at 23rd Jan. By contrast, the ratio between total nanoplankters+microplankters (both bacteriovorus and autotrophs) and metazoan biomass remained more stable (Fig. 5.10c), except at 23rd Jan, when a drastic increase near to 1:1 was observed in surface. Despite of this peak, values at surface and deep layers were around 4 and 0.2 respectively.

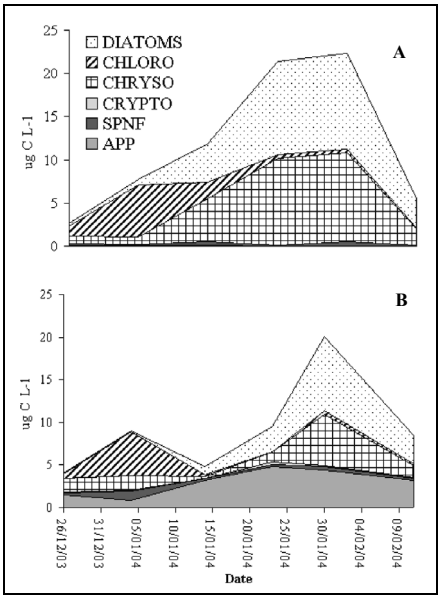

Figure 5.8. *Multiple area plot showing temporal variations of the biomass of phototrophic groups in surface (A) and at deep (B) layers respectively in Lake Limnopolar during the studied period. SPNF and APP indicate small phototrophic nanoflagellates and autotrophic picoplankters respectively.*

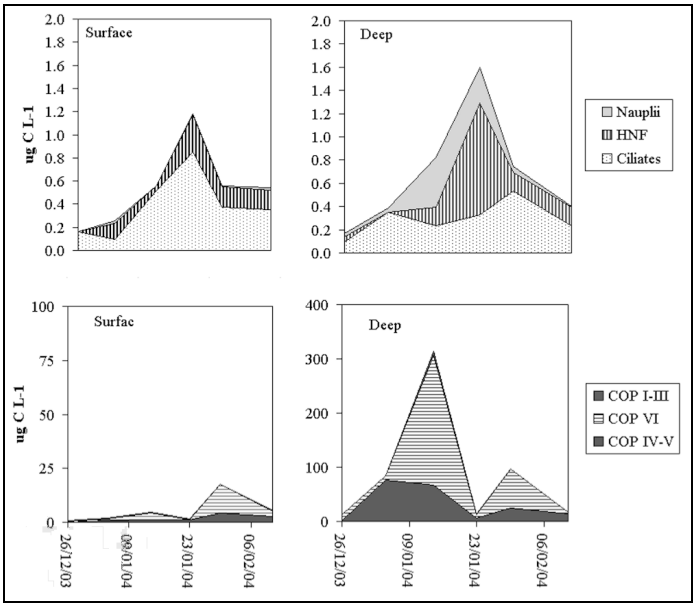

Figure 5.9. *Multiple area plot showing the seasonal dynamics of zooplankters biomass in Lake Limnopolar at summer 2003-04.*

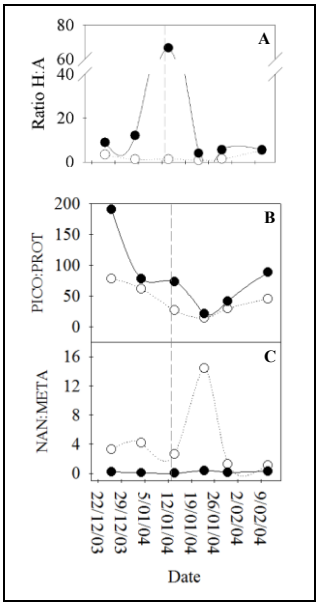

Figure 5.10. *Temporal variations in the pelagic food web structure represented as the biomass ratios of total heterotrophs versus total autotrophs (A); picoplankters versus protozooplankton (B); and total nanoplankters versus metazoan (C). White and black circles represent samples from surface and bottom layers respectively.*

5.3.7. Patterns of the plankton size spectra at summer 2003-04

In figure 5.11 and table 5.2 are shown the Pareto distribution of normalized size spectra and the underlying parameters of pelagic food web obtained at the different dates respectively. In general, a gap was observed for organisms sizing around 100 µm. The spectra were less truncated at the deep layer, particularly from mid to late January. The seasonal variation of regression coefficients and slopes obtained from the non-linear regression of these distributions are plotted in figure 5.12. Concerning to the goodness of fit, all regression coefficients (R^2) were always great than 0.9 and invariably significant ($p< 0.001$). The slopes values were always below -1, ranging from -1.65 to -2.34. Although trends of variation were quite similar at two depths, they were consistently less steep (less negative) at deep, except when the mixing of water column happened at mid February, just when slopes values coincided at two depths. With all, the average values of the intercepts and slopes were significantly higher and lower respectively in the surface layer compared to the observed at deep (Fig. 5.13). Observing the temporal evolution of the slopes, it is noticeable a progressive increase until mid January. After the outlet drainage, the slopes were steeper as a result of an increase of larger-sized plankton, mainly nano-sized pennate diatoms.

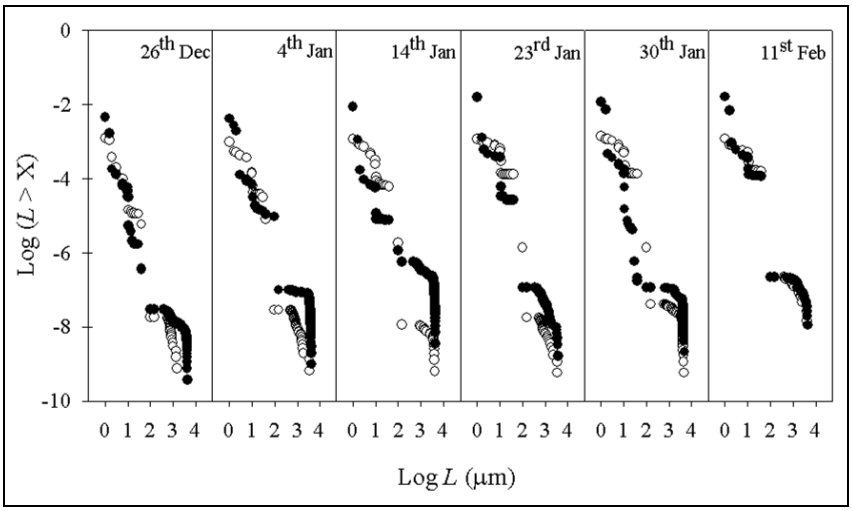

Figure 5.11. *Normalized biomass spectrum of planktonic community at surface (white circles) and bottom (black circles) occurring at different sampling dates in Lake Limnopolar. The plots cover the size range of the spectra from bacteria to copepods. Parameters of linear regression are given in table 5.1.*

Table 5.1. *Regression parameters obtained from the plankton normalized size spectra showed in figure 5.11. All regressions were significant at level <0.0001.*

	Surface			Deep		
	Slope	**Intercept**	**R^2**	**Slope**	**Intercept**	**R^2**
26th Dec	-2.008	-2.579	0.996	-1.433	-3.453	0.998
4th Jan	-1.890	-2.347	0.998	-1.295	-3.004	0.997
14th Jan	-1.818	-2.119	0.995	-0.999	-3.442	0.998
23rd Jan	-2.157	-1.621	0.995	-1.705	-2.320	0.999
30th Jan	-1.701	-2.026	0.998	-1.357	-2.786	0.996
11th Feb	-1.807	-2.656	0.997	-1.807	-2.494	0.996
Mean	-1.897	-2.225	0.996	-1.433	-2.917	0.997
SD	0.163	0.385	0.001	0.292	0.474	0.001

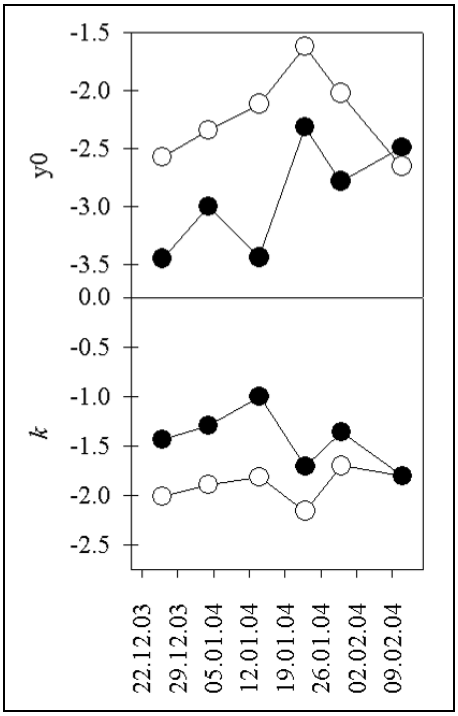

Figure 5.12. *Temporal variation of parameters extracted from Pareto fittings at surface (white circles) and deep (black circles) layers in Lake Limnopolar.*

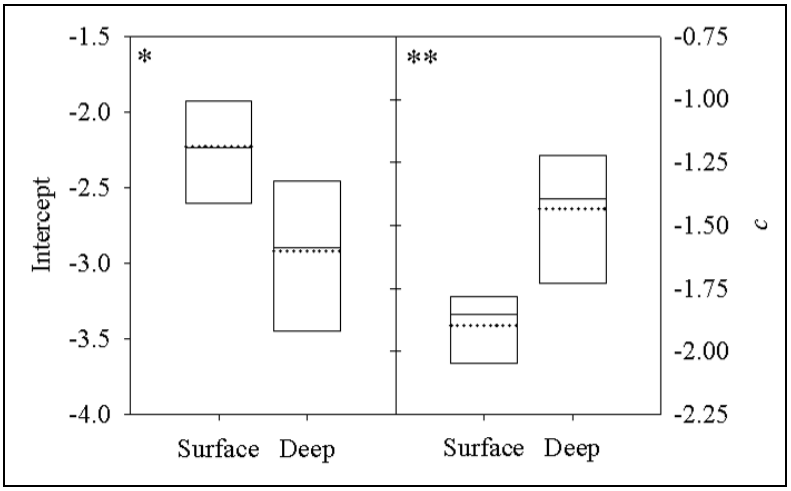

Figure 4.19. *Plot boxes showing the values of intercept and slopes of regressions at two depths enclosing data from 25 to 75% percentiles. The median and mean are represented by the solid and dashed lines respectively. Symbols * and ** indicate significant differences with p>0.05 and p>0.01 respectively.*

5.4. Discussion

This chapter provides the observation of seasonal patterns of planktonic populations in Lake Limnopolar during three climatologically contrasting summers. Particularly in summer 2003-04, it was possible to follow plankton dynamics throughout the course of thaw period, which embraced the more dynamic period of the lake. Based on nutrient and chlorophyll-*a* (Chl-a) levels, Lake Limnoplar can be classified as ultra-oligotrophic, which led to suppose a key role of microbial food web pathways. The ice melting should be an important episode in the lake for microbial dynamics since bacterial numbers are higher during this period. Besides, just before the ice melting, there is a development of phytoplankton populations, which can be associated to nutrient pulses. In such an oligotrophic lake, a nutrient release from melting, although to be low, likely explain this algal blooming, which must be favoured by the increase of light penetration because of the already narrowed ice cap.

Some generalities concerning dynamics of phytoplankton can be observed in our pigment analysis. The evolution of fucoxanthin concentrations followed very much Chl-*a* at summer 2003-04, inclusively when the onset of algal production took place. Fucoxanthin is present both in chrysophytes and diatoms, which does not allow to assert feasibly which taxa accounted for this peak. In summer 2001-02,

fucoxanthin increased during the ice-free conditions in parallel to the nanoflagellates (NF). However in 2003-04, the evolution of the pigment ratio did not follow exactly the nanoflagellates counts. It could respond to a major abundance of heterotrophic forms, but also to the adaptation of cells to the bright light experienced after the ice melting, which more probably involves a change in the cellular quota of these pigments (TANABE 2008). The diatoms assemblages observed in Lake Limnopolar are composed merely by pennate species. During periods of high turbulence, it is supposed for them a major role due to their higher sinking rates. In any case, seeing that thermal convection cease during the night, likely the motile and smaller cells remain in the upper layers more than these heavier diatoms. The development of chlorophytes, deduced from the amounts of lutein relative to Chl-a, might be in part explained also in these terms, as chlorophytes observed in Lake Limnopolar, at least during the ice-free periods, are mainly composed by larger cells.

At summer 2003-04, it is observed how the phytoplankton community varies closely related with changes of lake's physical structure. The period that preceded the lake's drainage was characterized by an increase of Chl-a concentrations with depth, which likely responds to the balance of nutrients and light availability. Thus, the stability of water column limits the nutrients diffusion to surface from the more nutrient-rich hypolimnion, which besides coincided with the lowest light availability. At this period, the autotrophic picoplankton was the more abundant phytoplanktonic group at deep layers. These subsuperficial peaks of picoplankters has been described yet in chapter 3, and observed also in others lakes from the region (e.g. Signy island, VINCENT 2000). It is known how the light which penetrates in ice covered lakes has a spectrum enriched in the blue-green region and is diminished in light at wavelenghts longer than 600 nm (VINCENT AND LAYBOURN-PARRY 2008). In our case, the inspection under the epifluorescence microscope of samples revealed the presence of phycoerythrin rich picocyanobacteria (APC) forming these populations. This light-harvesting pigment efficiently absorb light in the range of the blue-green wavelenghts, thus providing a potential capability for chromatic adaptation to the light regime occurring in Lake Limnopolar at this dates (CAMACHO ET AL. 2003a). On the other hand, a competitive advantage concerning a biomass-specific affinity for nutrients could be furthered here, thus enhancing the fitness of smaller cells (higher surface/volume quota) against the larger ones (lower surface/volume quota). This is supposed to be favored by the low turbulence of the water column, which increases the sinking rates of the large cells. This APC population showed a constant increase along time in the deep layers, except in January 30th, when the numbers of ciliate protozoa were higher, suggesting in this case a predatory control. The mixing forces accounting at the final of summer

probably eliminated this subsuperficial APC population by dilution in the water column.

However, the highest phytoplankton production is conditioned by the occurrence of turbulence in the water column. In Lake Limnopolar, most of the algal species are supposed to overwinter as resting stages until that environmental conditions allow them to develop. At summer 2003-04, the deepening of the upper mixed layer was likely accompanied by an upward diffusion of nutrients, which maybe started before by the occurrence of a diapycnal diffusion as discussed in the previous chapter. This fact, summed to the increase of light penetration in the water column, might explain the onset of phytoplankton production. In any case, it seems that the time just before the break of the ice cover encompasses the better conditions for the algae enhancement. It is also the case of Otero Lake in the Peninsula Antarctica (MATALONI ET AL. 2000). There, these authors propose high hydrologic residence times to explain the fast algae development. Just before the commencement of the ice thaw, the outflow in the catchment is minor and therefore the advective losses are more reduced. The intense water discharge occurring in Lake Limnopolar at mid January of 2004 did not imply, however, a significant change in the plankton biomass, which apparently is in disagreement with this idea. These changes in the residence time are supposed to impact differently in organisms depending of its generation time. For instance, the zooplankton is kwon to be more influenced by this compared to phytoplankton (BUM AND PICK 1996).

A part of the phytoplankton assemblage observed in Lake Limnopolar appears to be incidental. It is the case of diatoms, which are totally represented by pennate forms (including large forms) which probably have an allochthonous origin. They may come from the benthic communities growing in the stream inlet, from lake's bottom, or even from the Lake Somero. It could be consequence of an intense erosion taking place during the thawing and/or rainfall events, which should be favored by the high catchments/size relationship of the lake. As mentioned previously, the silica wraps of these diatoms determine high sinking velocities, therefore, the turbulence endures their maintenance in the water column. This large phytoplankton may also originate, in part, from the flora attached to the benthic mosses. Later has been observed in Moss Lake (Signy Island) by PRIDDLE AND DARTNALL (1978), which showed a major reallocation of epiphytic species previously attached to sub-aquatic mosses during the summer mixing.

Differently to the large phytoplankton, nanoflagellates crisophytes show an active motility that allows them to cope with sinking forces. It would explain why they have a higher role in the assemblage at the commencement of summer.

However, the dominant species differed with time. Thus, species of genera *Pseudokephyrion* and *Ochromonas* peaked in deep layers during stable conditions, whereas *Chrysoccocus* spp., although to be always the dominant, were even more abundant during the onset of phytoplankton production, just when turbulence increased. The former are known to be mixotrophic organisms, namely, they are facultatively heterotrophic (both phagotrophic and/or osmotrophy) in the absence of adequate light. This represents a beneficial strategy in unproductive ecosystems since it provides the capability to obtain carbon by means of both fagotrophy and autotrophy. Therefore, it confers to these species an advantage compared to those only able to achieve one of these processes (LAYBOURN-PARRY ET AL. 2005). The later could be the case of *Chrysoccocus* spp., which as mentioned before dominated in the water column when turbulence starts.

Aside from the differences in the copepods abundances, it is also interesting to note the inter-annual variation of the population age structure. Compared to previous years, our results demonstrate a deferred maturation occurring at summer 2003-04. It can be due to a delay in the hatching events or a diapause of any copepoid stage, which could be a consequence of the persistence of the ice cover. As commented before, algal bloom occurs just before the ice thaw, which likely provides the better opportunity for copepods of high food quantity and quality. It is interesting to consider here which SEEBENS ET AL. (2009) observed during a long-term study involving copepods (*Cyclops vicinus).* In that case, *Cyclops vicinus* was confronted with a variable phytoplankton bloom phenology. As a result, a delay in the maturation of copepods was the strategy for guarantee the match with the highest food availability. SEEBENS and co-workers also observed that the higher survival rates were dependent on the phytoplankton bloom timing, in such manner that these rates were higher at years in which the phytoplankton bloom occurred earlier. As point out these authors, an important subject should be to appraise if these strategies would be flexible enough to cope with a future climate warming.

We can try to assert also the relative role of trophic and non-trophic processes affecting plankton dynamics. In contrast to protists, the vertical distribution of viruses like-particles (VLP) and bacteria during 2003-04 summer showed a marked vertical heterogeneity. Also, both the absolute abundance of VLP and the Viruses:Bacteria abundance ratio (VBR) showed a significant correlation (R^2=0.84, $p<0.01$; R^2=0.92, $p<0.01$) with copepods densities. In spite of the low sampling resolution, this apparently agrees with observations situating the higher abundances of bacterial and viruses in the thermocline of lakes (WEINBAUER ET AL. 1995, DRAKE ET AL. 1998, BETTAREL ET AL. 2003). Likely, there are different

mechanisms modulating in combination this pattern. In the case of bacteria, factors such as temperature and nutrients availability, or even top-down forces, might be accountable. The higher alkalinity of water observed at deep layers suggests, for instance, more elevated photosynthetic rates, which would be due to the presence of the mosses carpet.

This primary production is supposed to trigger the release of dissolved organic carbon, which may fuel bacterial production. In other respects, regardless of depth, bacterial abundances increased after the ice thaw. When the lake is covered by the ice, the upward diffusion of nutrients is maybe the only support for bacterial production. By contrast, after the thaw, bacteria can be also subsidized by allochthonous carbon originating from the catchment. The later is further suggested by the observed parallel increase of CDOM concentrations at the final of January (see figure 4.33). In our opinion, this carbon could have a pivotal role in maintaining the whole pelagic production. The idea that freshwater ecosystems are predominantly systems that respire more organic carbon than they produce has been argued by COLE (1999), who sign a general tendency towards a net heterotrophy in lakes as them become more oligotrophic. This same idea has been confirmed once in posterior experiments (PACE ET AL. 2004, CARPENTER ET AL. 2005). Besides, PACE ET AL. (2007) observed afterwards that allochthonous resources can be relatively more important in small lakes, such as in our case, because loadings decline as the perimeter to area ratio declines with the increase of lake size.

The slight changes observed in bacterial abundances until late January does not imply necessarily a nutrient limitation. This may respond to a close balance between production and mortality process. The VLP showed abundances in average one order of magnitude higher at deep layers, which are yet in the range of the reported for other Antarctic lakes (LE ROMANCER ET AL. 2007 and articles cited therein). This suggests that these viruses are strongly associated to bacterial production; or at least to their abundances, given that the viral infectivity is a host density-dependent process. However, it has been occasionally suggested that a strong co-variation of bacteria and viruses hints a great domain of bacteriophages on the assemblage (BETTAREL ET AL. 2003b), which could not be our case because we observe notable variations along time of the viral-bacterial ratios (VBR; Fig. 5.3b). Unfortunately, our counts are not able to discriminate which proportion of viruses are bacteriophages or, by contrast, host other organisms such as phytoplankters. The other possible mechanism regulating bacterial abundances can be a top-down control exerted by bacteriovorus, which in our case is further suggested by the decline observed in bacterial numbers just when nanoflagellates peaked.

The body-size spectra obtained at summer 2003-04 provides insights on trophic interactions occurring in the lake. For instance, it gives rather information about the assembly of plankton community. Here, we can conjecture if endogenous factors (i.e., predation, competition, etc,.) underlie the dynamics observed or, by contrast, they have a subordinate role. In our case, the spectra resulted highly reactive to the physical changes occurring in the lake. This can be traced observing the changes in their underlying parameters (Fig. 5.12). The slopes of normalized size spectra ranged between -1.71 and -1.96. In general, these values are steeper than the expected for a steady state considered in the "linear biomass hypothesis" (MARQUET ET AL. 2005), thus demonstrating low exploitation efficiencies. This brings forward the lack of the steadiness of this pelagic community, which in our opinion responds to the tight coupling between biological dynamics and the environmental instability. As period advanced, the spectra became flatter, thus reaching slope values of around -1.7 at the final of January, which is produced by the enhancement of the nano and microphytoplankton size fractions. At mid February, slopes decreased in both depths coinciding with the higher bacterial abundances. The shallower slopes, which could mean an increase of the predator-prey biomass ratio, can be interpreted as a higher efficiency of energy transfer along food web. On the other hand, smoother size spectra have been showed to reflect more diverse plankton communities (GAEDKE ET AL. 2004).

Irregularities in the body-size distribution seem to prevail under unstable conditions such as the observed during algal blooms (GASOL ET AL. 1991, TITTEL ET AL. 1998). These irregularities are reflected in our case by the waviness of the spectra. This is provoked by the development of less edible algae such as long pennate diatoms and filamentous forms. Concerning the gaps observed in the spectra, we consider that they are not imputable to incomplete censuses or bias in our analysis rather than to discontinuities attributable to inherent properties of the lake's food web. Indeed, this seems to be a regular feature in lakes (HAVLICEK AND CARPENTER 2001). In any case, it is not clear what mechanism underlies the occurrence of these gaps. It is possible that these breaks respond to a trophic cascade due to a strong size-selective predation (CARPENTER AND KITCHELL 1993). In this sense, given that these gaps match well with the size of rotifers, which are rarely observed in the lake, our discussion concerning to their low abundances can be considered here (see general discussion in chapter 10).

The biomass ratios between different trophic levels (Fig 5.10) offer further insights on food web functioning. For instance, the ratios between the heterotrophic and autotrophic carbon pools agree with the widespread opinion that low-productive

systems tend to be net heterotrophic (COLE 1999, COLE ET AL. 2000). In our case, biomass is greatly allocated in bacteria and copepods. The occurrence of high pools of zooplankton in oligotrophic conditions is in conformity with the observed by JEPPESEN ET AL. (2003) in a latitudinal study that covered a 500-fold gradient of phosphorus concentrations. This high copepods biomass leads to expect that a substantial carbon transfer occurs from microbial components to the higher trophic level. By contrast, the standing stocks of bacteriovorus in the lake are frequently low despite to their elevated growth rates, therefore, it is possible to think that copepods are efficiently controlling their populations, which effect supposedly cascade to pico-sized organisms. The temporal uncouple observed between the abundances of copepods and protist reinforces this idea. Our hypothesis is that pathways of carbon transfer in the pelagic compartment may vary depending of the plankton size structure. Thus, a higher domain of picoplankters might lead a higher turnover of nutrients through the microbial loop. By contrast, if large plankton dominates, biomass might accumulate in the benthic compartment due to the higher sinking rates. Nutrients recirculation would be lower in the latter case because part of this plankton is few edible as demonstrate our experiments (see chapter 6).

We also observe a spatial segregation of carbon pathways in the lake. Microbial loop pathways apparently dominate in deeper layers, where the DOC released by benthic mosses likely fuels them. At this depth, we observe the higher VLP and bacterial abundances as well the higher CDOM signal. This has been also observed in other Antarctic lakes (SAWSTROM ET AL. 2007), suggesting the occurrence of direct causal links between these parameters. In the bottom is also observed the less negative slopes of the body size spectra, thus indicating a higher efficiency of biomass transfer. With all, these slopes point out the existence of few weak trophic links (i.e., low connectance). Following the idea of PINNEGAR ET AL. (2005), a low connectance probably allows to the system be more dynamically stable, thus recovering more rapidly following disturbance. It is because multiples stable equilibriums are more possible when there is a low connectance (CHASE 2003). However, a high productivity and low disturbance are both trends lacking in Lake Limnopolar that probably are required to allow this dynamic stability.

Concerning the vertical movements of zooplankton, it has been proposed that a relaxation in circadian rhythms may occur at high latitudes due to the local variations of the day-night cycle, thus reducing the vertical movements (HANSSON ET AL. 2007). Additionally, the underwater illumination beneath the ice must be too much low to produce a significant response in the population. Still, we observe how a part of the population of *Boeckella poppei* undertakes a nocturnal vertical

migration (DVM). A hiding behaviour can be discarded here by the absence of predators. This can be explained by a nektobenthic behaviour, which would be in conformity with the reported for this specie in other sites of the region such as in Hope Bay (IZAGUIRRE ET AL. 2003) or Signy Island (HEYWOOD 1970). On the other hand, given that the development is temperature dependent, it is also possible that this copepod establishes better in the warmer waters during the inverse stratification. As state BOLLENS AND FROST (1991), later may suppose a demographic advantage since the development of eggs is delayed in surface cooler waters. This agrees with our observations showing mature stages of *B. poppei* mainly confined to the deep layers. An interesting idea is that this diurnal migration may determine that grazing and food metabolization, and therefore the subsequent release of nutrients, take place at different depths. So, by means of this nocturnal ascent, the copepods would transport nutrients from deep layers to the nutrient-poor surface. This is speculative and not sustained by field observations, however, it is a phenomenon described in lakes from temperate regions (SAWATZKY ET AL. 2006). Some authors have defined this as a 'biological pump' which supply in part the production in the upper layers with resources originated from the deeper nutrient-reach strata (PILATI AND WURTSBAUGH 2003; CAMACHO 2006b).

In summary, we have shown how the pelagic production in Lake Limnopolar is regulated by the co-variation of temperatures and light availability, but also by an increase of nutrient fluxes. We propose that the nutrient content in lake is subsidized by inputs from the catchment, but also there is a reprocess of internal pools due to a density-driven water transport. Our outcomes, akin to other observations (ADRIAN ET AL. 1999; PARK ET AL. 2004), demonstrate how variations in the timing of ice cover drastically affect the dynamics of plankton community. The year-to-year variability observed in the region does not allow then to expect regular patterns. With all, our findings indicate a predominant role of heterotrophic pathways (i.e., microbial loop) in Lake Limnopolar, which seem to be efficiently canalized, at least during some periods, to the metazoan production. Even so, given the efficiencies observed in the biomass transfer, it appears that the lake is not able to support more trophic levels than those observed. Accordingly, the mechanisms conducted to make shorter the food web such as omnivory and myxotrophy (which means that one can to profit from more than one trophic level) are likely favoured as they improve the efficiency of food web functioning. A study addressed to explore some of these trophic interactions is showed in the next chapter.

6. Effects of grazing and nutrient enrichment in the microbial food web of Lake Limnopolar

6.1. Introduction

Antarctic lakes are simple environmental system suitable for testing basic principles of functional ecology. Biotic interactions such as predation and competition (both top-down mechanisms) or resources availability (bottom-up mechanisms) are factors controlling food web structure in temperate areas (CARPENTER AND KITCHELL 1993). There is some literature sustaining that merely environmental forces control Antarctic ecosystems (KREBS 2001, MCKENNA ET AL. 2006). The fact that lakes from Byers support plankton in truncated food webs (see chapters 3 and 5) holds in part this idea. However, the occurrence in some periods of low stocks of protists, which is noteworthy in view of their great potential for rapid growth (HANSEN ET AL. 1997), induce us to suspect the occurrence of a metazoan-driven control of their populations. This possibility has been put forward in a study performed by HANSSON (1992), who compared the response of phytoplankton to phosphorus increase in Swedish lakes, with functionally three trophic levels, with that of Antarctic lakes (functionally two trophic levels). HANSSON found differences showing that at low or moderate productivity, besides the effect of nutrients, food chain composition had a crucial impact of the biomass development of planktonic algae. The two-level systems studied by HANSSON in Antarctica were composed of primary producers and crustacean grazers, which are quite similar to those observed in Byers. These planktonic crustaceans are broadly known to control prey populations and release nutrients (DODDS 2002, BERMAN AND BRONK 2003). These observations have driven to some researchers to believe that top-down mechanisms might play a major role in Antarctic lakes, at least during short time periods (e.g. MATALONI ET AL. 2000, CAMACHO 2006a).

Beyond their trophic structure, the productivity of Antarctic lakes, which commonly rang from ultra-oligotrophic to oligotrophic, is limited by the availability of nutrients. Consequently, the food webs' structure is expected to be sensitive to nutrient inputs. Indeed, the influence of the nutrients enrichment on phytoplankton has been experimentally touched upon in the MacMurdo region (PRISCU 1995), showing to be a major responsible of growth limitation. The phosphorus has been traditionally considered responsible to constraint phytoplankton growth by itself, however, a co-limitation involving nitrogen may also occur. This has been verified in the northern hemisphere (CAMACHO ET AL. 2003b; DZIALOWSKI ET AL. 2005; LEWIS AND WURTSBAUGH 2008), and could be also regular in Antarctic lakes (PRISCU 1995, BELL AND LAYBOURN-PARRY 1999). In this sense, the isolation of polar regions implies little human influence and a limited nitrogen supply via atmospheric deposition. In other respects, our findings indicate that carbon fluxes in

lakes from Byers largely take place through microbial food web pathways. In such cases, the bacterial edibility may result in an important regulatory mechanism, which furthermore is kwon to differ depending of trophic status (MATZ AND JÜRGENS 2003, THELAUS ET AL. 2008). On the other hand, bacterioplankton in Antarctic lakes has been found to be co-limited by phosphorus and carbon (SAWSTROM ET AL. 2007), which as suggested in chapter 3, might also occur in lakes from Byers.

Both quantitative and qualitative impacts of top-down and bottom-up mechanisms depend of the food web architecture (PERSSON ET AL. 2001, HENRY ET AL. 2006). For instance, factors such as the prey edibility or the resource utilization affect the extent at which the trophic cascade propagates through the food web. Further, the nutrient status of the community may damps or enhances these cascade effects. BERTILSSON and co-workers (2003) observed a good example of a structuring mechanism in Arctic lakes relying on the prey size-selectivity. In that case, different compositions of the zooplankton community (which varied in their relative dominances of large cladocerans, copepods or fairy shrimps), produced variations of bacterial and phytoplankton abundances. These experiments showed that cladocerans predated over all prey types, whereas copepods grazed preferentially on larger cells (both phytoplankton and bacteria). Moreover, there is the example of copepods from some New Zealand's lakes, which overcome cladocerans when they predated on protozoa (BURNS AND SCHALLENBERG 1996, 1998). In this case, the copepod *Boeckella hamata* depressed ciliates population, whereas *Daphnia carinata* predated mainly on picoplankters. It must be noted that a domain of copepods (*Boeckella poppei*) and low nutrient loads are also regular features of lakes from Byers (e.g., Lake Limnoplar). Also remarkable observations were made by ELSER AND GOLDMAN (1990), who stated a depletion of grazing pressure in low productive lakes with a zooplankton assemblages dominated by copepods. For these authors, it is because copepods are less efficient grazers compared to large-sized cladocerans.

The trophic interactions occurring in Antarctic lakes, which may comprise both biotic and abiotic feedbacks, are not yet totally understood. Hitherto, only few studies involving field manipulations have dealt with the plankton food webs functioning of Antarctic lakes (ALLENDE 2009). The main goal of this chapter has been to explore the potential capability that biological interactions have to regulate the structure of planktonic communities, in spite of the strong physical control. For this propose, we carried out three different microcosm experiments that involved the manipulation of metazooplankton abundances and nutrient concentrations. They were performed at three consecutive summers (2002, 2003 and 2004) with water of

Lake Limnopolar. Additionally, we show here data on the isotopic carbon signatures ($\delta^{13}C$) of different compartments of the lake to provide a time-integrated measure of food web relationships. The results obtained in this isotopic analysis are discussed jointly with the experimental outcomes.

6.2. Methodology

6.2.1. Sampling for isotopic ($\delta^{13}C$) determination of different lake compartments

A fractionated sampling was conducted at February 2002 in Lake Limnopolar to identify the delta ^{13}C values of different pelagic and benthic compartments. The samples were obtained as follow. The seston was fractioned into different size classes: >250 µm, 250-150 µm, 150-50 µm, 50-20 µm, and <20 µm. Moreover, particulate material from the inlet water were also obtained, which consisted of fractions >200 µm, >150 µm, and >50 µm. The copepods retained in filters were separated for analysis. The benthic fauna (*Branchinecta gaini, Parochlus*) was collected with a net and subsequently individuals were isolated under a dissecting scope. Aquatic mosses and sediments were collected with a dredge from the central point of the lake. All samples were stored at -20ºC until analysis. Before analysis, samples were dried and ground to powder. For the analytical processing of data we conducted as explained in section 2.1.9.

6.2.2. Feeding experiments

To determine the prey size preference of *Boeckella poppei*, individuals were incubated in vials with water from Lake Limnopolar filtered by GF/F filters containing a solution of spherical latex beads (Bang-Labs Inc.) of 5, 10 and 20 µm diameter. These dimensions represented well the dominant size classes of planktonic microorganisms observed in pre-screened samples of the lake. During incubations, the vials were turning gently twice to keep microspeheres in suspension. An adequate incubation time of 20 min was previously determined in time-course experiments. After this time, formaldehyde (4% final concentration) was added to the samples for stop the experiment. Once in the lab, copepods were treated with a concentrated solution of acid lactic. This procedure bleached their pigmented carapace, facilitating the visualization of microspheres inside the guts but allowing

that beads remained undamaged. The number of microbeads inside the guts was counted for a total of 80 individuals. The grade of selection for each copepod stage was measured using a dimensionless electivity index (ε_i; CHESSON 1983), which is obtained from the following equation:

$$\varepsilon_i = 2\left\{\frac{\ln\left[\left(n_{i0} - r_i\right)/ n_{i0}\right]}{\sum_{j=1}^{m} \ln\left[\left(n_{j0} - r_j\right)/ n_{j0}\right]}\right\} - 1 \qquad \text{(Equation 6.1)}$$

where n_{i0} is the number of microspheres of type i added to incubation bottle, r_i is the number of microspheres of type i counted in the guts of copepods, and m is the number of microspheres types (m=3). This index is unaffected by the relative abundance of food items and varies from -1 for negative preference to +1 for positive preference. The values equal to 0 indicates then a none selective feeding towards the prey i.

Analogous experiments were conducted at January 2007 to test the predatory behaviour of protists upon picoplankters. In this case, fluorescent microspheres 0.5 and 1 µm of diameter (Fluoresbrytes, Polysciences) were added to 125 ml of water from Lake Limnopolar to constitute roughly 30 and 1.5 % respectively of natural picoplankton abundances. It was made considering that picoplankters numbers (HPP + APP) observed in previous years in Lake Limnopolar ranged from 8 x 10^5 to 1.5 x 10^6 cell mL^{-1}. Following these premises, a time-course experiment was conducted for lag times of 0, 10, 30, 60 and 90 min. The preparation of slides for the microscopic counts of beads was made following the procedure described in section 2.3.3. Filtration rates were calculated by means of a clearance rate (CR) as described in equation 6.2, which is an estimation of the volume of water cleaned up of microspheres per time. In the equation, F_{ing} and F_t are the particles ingested after time t and total particles added to sample respectively. Only the data within the time interval fitting with a linear regression were used for calculations.

$$CR = \frac{F_{ing}}{F_T} \cdot t^{-1} \qquad \text{(Equation 6.2)}$$

6.2.3. Setting of microcosm bioassays

Three short-term microcosm experiments were conducted in Lake Limnopolar at consecutive summers, lasting 10, 13 and 7 days respectively. They were ran during open water conditions, the first (experiment-I) at February 2002, the second (experiment-II) in February 2003 and the third (experiment-III) at February 2004. Logistic reasons made convenient to incubate samples near the camp with continuous flowing water in a stream distanced approximately 2 km from the lake (Fig. 6.1). During the course of the experiments, carboys were stirred every day to minimizing algal growth on the enclosure walls.

For **experiment-I**, PVC carboys of 5 L (Cubitainer®) were filled with water from 0.5 m depth of the lake. Experiment consisted in a two-by-two factorial design in which nutrient enrichment and metazooplankton presence/absence were crossed. Nutrient (nitrogen and phosphorus) were added both jointly and separately to determine which nutrient, if any, might be limiting for growth. Accordingly, four different conditions were assayed as follows: (C) a control containing lake water without nutrient additions; (+N) lake water supplied with NH_4NO_3 final concentration 60 µM N; (+P) lake water supplied with NaH_2PO_4 final concentration 4 µM P; and (+NP) lake water supplied with NH_4NO_3 final concentration 60 µM N and Na_2HPO_4 final concentration 4 µM P. Each of these nutrient conditions were treated as follow:

1) lake water previously filtered through a 150 µm size nylon mesh to retain metazooplankters (referred as treatment –GRAZ in figures and text).
2) a 30-fold increment of zooplankton abundance simulating the high zooplankton abundances detected in lake during this summer (referred as treatment +GRAZ in figures and text).

To increment zooplankton abundances, the concentrated retained in the nylon meshes was resuspended in the carboys. To this way, the experiment resulted in 8 different conditions assayed by triplicate (24 enclosures).

Differently to the former, **experiment-II** was designed to grade mesoozooplankton densities. In one of the treatments, water was pre-filtered through a nylon mesh of 50 µm to assure the removal of all metazoan. The other treatments were pre-filtered by a mesh of 150 µm to establish a set of scaled copepod densities, namely 0x, 1x, 2x, 4x, 8x, 16x and 32x times the ambient abundances of *B.poppei*. By following this procedure, we intended to remove respectively only large

(filtration by 150 µm) and both large and small zooplankton (filtration by 50 µm). To establish the gradient, the copepods concentrated in the 150 µm net were stocked in carboys at the densities mentioned above. After that, two different nutrient treatments were imposed to each of them as follow:

1) A total of 24 enclosures, referred to as +NPSi, received a nutrient supplement (NH_4NO_3, 60 µM-N, NaH_2PO_4 4 µM-P and Na_2SiO_3 100 µM-Si final concentration).
2) A total of 24 enclosures, referred as –NPSi, were not fertilized.

Differently to the former experiment, in this case the silica was added as $Si(OH)_4$ to prevent a possible growth limitation of diatoms and/or silica capsulated chrysophytes. The experiment resulted then in 16 different conditions assayed by triplicate (48 enclosures).

Figure 6.1. *A picture showing the PVC carboys used in the experiments. For logistic reasons the incubations were carried out near to the camp, sited approximately 2 km to the lake, in a stream with continuous flowing water.*

In **experiment-III** the samples were also fractionated into various size classes by filtration. In this case, three different sets were established as follow: a) non filtered water; b) water filtered through a 150 µm mesh; and c) water filtered through a 50 µm mesh. The different filtration treatments were dispensed into the 5 L carboys to start the experiment. Hereinafter, aliquots were taken from the microcosms at intervals of 12-24 h during a week to determine the densities and

growth rates of different microbial populations. In this case, the results obtained were transformed to biovolume as is described in section 2.4. The 3 different conditions were assayed by triplicate (9 enclosures).

For protists, the rates of production (P_i, cells ml^{-1} h^{-1}) and losses due to predation (G_i, , cells mL^{-1} h^{-1}) were estimated using the following equations:

$$Pi = \left(\frac{\mu_{<50\mu m}}{\mu_{GRAZ}} \right) \cdot N_{i-1} \cdot \left(e^{\mu GRAZ} - 1 \right) \quad \text{(Equation 6.3)}$$

$$Gi = \left\lfloor \frac{\mu_{<50\mu m} - \mu_{GRAZ}}{\mu_{GRAZ}} \right\rfloor \cdot N_{i-1} \cdot \left(e^{\mu GRAZ} - 1 \right) \quad \text{(Equation 6.4)}$$

where $\mu_{<50}$ is the mean growth rate (h^{-1}) of protist after 12 h in the carboys filtered by 50 µm, μ_{GRAZ} is the same for the none filtered carboys, and N_{i-1} is the population density at the initial time. These equations are based in the formulation used by NAKANO ET AL. (2001), which assume that predation is totally avoided in carboys pre-filtered by 50 µm and their growth rates are equal in different treatments, at least during the initial period of the incubations (12 h).

6.2.4. Data analysis

The biological parameters obtained in the experiment I and III were also computed using the standard exponential growth equation:

$$\mu = \ln\left(N_{t1} / N_{t0}\right) \cdot t^{-1} \quad \text{(Equation 6.5)}$$

where t = time in days, N_{t1} = Population abundance at time 10, N_{t0} = Population abundance at the start of the experiment. In the experiment-II we used the log transformation of copepods abundances to linearize the relation and to get better homogeneity of the error variance. In the experiment-I, a two-way analysis of variance (ANOVA) was performed to assess zooplankton and fertilization effects, as well zooplankton-fertilization interactions. Because most of data were not normally distributed, nonparametric analyses were used as a rule. When significant dissimilarities were observed for any variable, pairwise contrasts (Scheffé test) were made to determine which treatment ensued different. In all cases the statistical

significance was satisfied at level of p=0.05. In the experiment-II, Spearman correlation analyses were carried out to test the relationships between variables. All the statistical tests were performed in the software SPSS 15.0 for Windows.

6.3. Results

6.3.1. Isotopic signatures in the food web of Lake Limnopolar

The $\delta^{13}C$ values from 15 compartments of the Lake Limnopolar are listed in table 6.1. The aquatic mosses (*Drepanocladus longifolius*) showed the more depleted value of –29.3, followed by the seston incoming from the catchment (-28.4). For the later, neither differences on carbon fractionation was observed depending of which filter size was used, thus suggesting that the incoming seston composed mainly of particles higher than 200 µm. Notable differences were observed by contrast among the pelagic fractions. In this case, the finest sizes (i.e., <20 µm and 20-50 µm) showed a more depleted values compared to the size fractions up to 50 µm. Aquatic mosses and the fraction between 20-50 µm were more enriched in ^{13}C than the copepod *Boeckella poppei*, which showed a $\delta^{13}C$ signature quite similar to the size fraction below 20 µm. On the contrary, the signature of the fairy shrimps (*Branchinecta gaini*) was close to the delta value of the lake sediment. The tissues of the *Parochlus* larvae showed similar values to those observed for the fairy shrimp although slightly depleted.

6.3.2. Grazing rates and prey size selectivity of zooplankters

The grazing activity of nanoflagellates, measured by using fluorescent microspheres (Fig. 6.2), displayed notable differences between heterotrophic (HNF) and pigmented forms (PNF). The time course of their grazing activities are shown in figure 6.3. For HNF, the particles uptake showed a progressive increase that fitted with a linear regression (R^2=0.87; p=0.029) until 30 min. Thereafter, the clearance activity declined probably by the egestion of beads. By contrast, the use of particles by PNF was not so obvious. In this case, only a slight increase of the clearance activity was observed that peaked at 30 min. Ciliates were seldom observed during the microscopic inspection of samples. It is for this reason that it was not possible to infer significant results for them in this experiment. More probably, the positive

pressure exerted during the filters preparation preferentially produced a rupture of ciliates cells, thus creating an important bias in counts.

Table 6.1. *Isotopic fractionation (expressed as $\delta^{13}C$) in different pelagic, benthic and inlet compartments of Lake Limnopolar.*

Compartment	$\delta^{13}C$	
	Mean	**SD**
Aquatic mosses	-29.29	0.46
Filamentous algae	-22.46	0.22
Lake seston < 20 µm	-25.69	0.18
Lake seston 20-50 µm	-27.68	0.22
Lake seston 50-150 µm	-22.01	0.78
Lake seston 150-250 µm	-24.63	0.96
Lake seston > 250 µm	-21.66	0.76
Total lake seston	-25.21	0.53
B. poppei	-25.48	2.54
Branchinecta gaini	-21.50	0.76
Parochlus	-22.98	0.54
Lake sediment	-19.92	1.89
Inlet seston >200 µm	-28.59	0.23
Inlet seston >150 µm	-28.36	0.23
Inlet seston >50 µm	-28.24	0.19

The clearance rates of nanoflagelates were obtained with a linear regression by fitting the data of the time interval before that grazing saturated. The purely heterotrophic nanoflagellates showed a significant higher activity when compared to the others (t-test, $P < 0.05$). Thus, the clearance rate in the case of HNF was 2.14 (±0.30 SD) nL $cell^{-1}$ h^{-1}, whereas PNF displayed mean value of 1.02 (±0.38 SD) nL $cell^{-1}$ h^{-1}. Concerning the size selectivity of particles, heterotrophic flagellates with a length smaller than 5 µm clearly showed a total preference for the 0.5 µm beads (ε_i = 1). By contrast, the larger heterotrophic flagellates (~10µm) preferred bigger particles as they showed a selectivity index of -0.63 and 0.63 for 0.5 and 1 µm beads respectively. These larger flagellates did not show in any event clearance rates significantly different to the smaller ones.

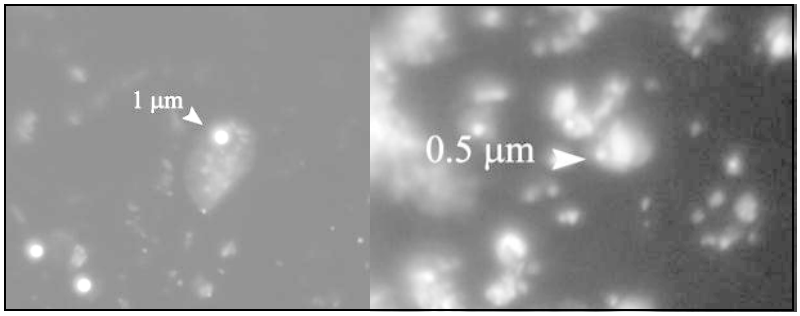

Figure 6.2. *Microphotographs showing ingested beads by different nanoflagellates.*

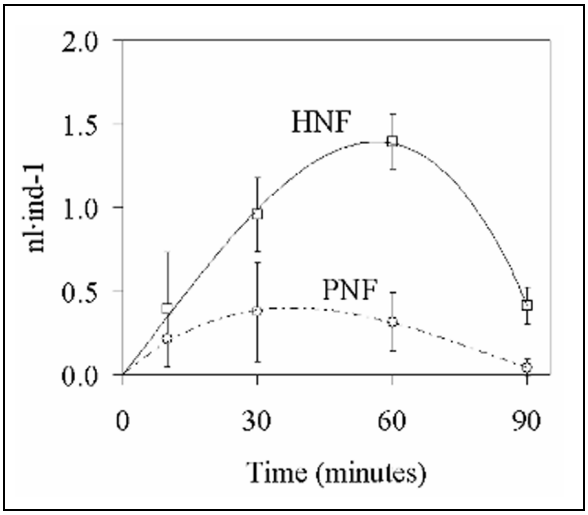

Figure 6.3. *Evolution of grazing over beads exerted by both nanoflagellate groups from Lake Limnopolar.*

The usage of beds as food by copepods was also verified (Fig. 6.4). A selectivity index (ε_i) for each particle type was obtained for the different age stages, demonstrating that no particle size was invariantly ingested by the different copepods ages. As shown in figure 6.5, a predilection for small beads (5 µm) was noted in the immature stages (both I-III and IV-V age clusters), whereas medium beads (10 µm) were the preferred by adults (age VI). The larger particles (20 µm) displayed also a positive relationship with the copepod size; but in general, they were avoided by all copepods stages.

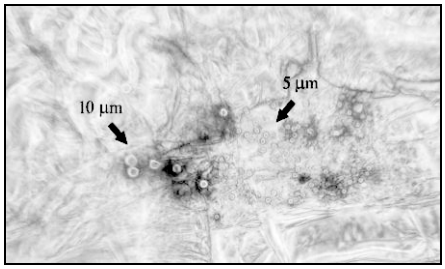

Figura 6.4. *The gut content of Boeckella poppei showing some ingested particles.*

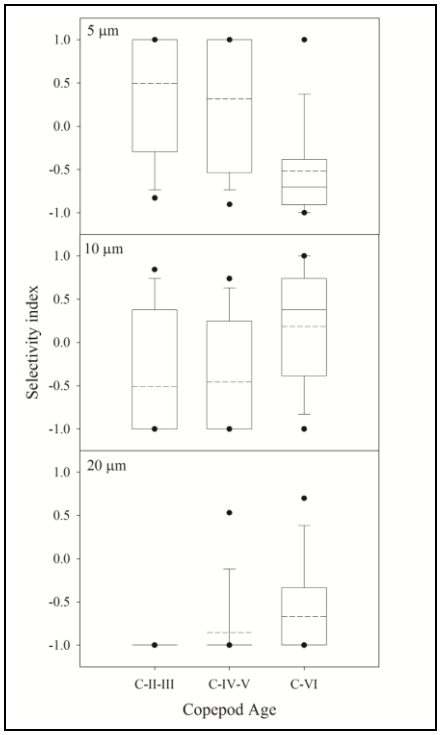

Figure 6.5. *Box plots of the selectivity indexes obtained in the different age states of Boeckella poppei for each bead sizes. Points indicate 5th and 95th percentiles, boxes delimit 25% and 75% percentiles, and solid and dash lines are median and mean respectively.*

6.3.3. Experiment I

6.3.3.1. Zooplankton abundances and nutrient availability

The copepod *Boeckella poppei* was the only macrozooplanktonic specie found in the water column of Lake Limnopolar at the time of the bioassay, showing densities in surface waters up to 2 ind L^{-1}. Adults mainly composed population when experiment started (89 %), whereas juvenile stages were less common (9 % copepodites and 2 % nauplii). The experimental manipulation supposed copepods densities around 50 ind

L^{-1} in those treatments augmented. With regards nutrients, a total exhaustion of soluble inorganic nutrients (N and P) occurred during incubation, both in the controls, where soluble N and P decreased to undetectable levels at the end of the bioassay, as well as in the treatments in which a single nutrient was added, in which the other was completely exhausted during the incubation. The same pattern was found regardless of the presence or absence of zooplankton. This shows that the single addition of a nutrient in excess resulted in the induction of limitation by the other. However, in the +NP treatments, where both nutrients were added, final dissolved phosphorus concentrations were quite similar either with or without zooplankton, whereas the treatment with zooplankton showed a 37 % higher concentration of dissolved ammonium.

6.3.3.2. Effects of predation and resource availability on the microbial loop components

Both abundances and growth rates of microbial loop members were determined at the final of experiment (Fig 6.6 and 6.7 respectively). A two-way ANOVA was made in order to look for significant differences depending of zooplankton abundances and/or nutrients availability. The results of the ANOVA are shown in tables 6.2 and 6.3 respectively. Concerning to the heterotrophic picoplankton (HPP) and regardless of the nutrient additions, in all treatments in which zooplankton was present they showed significantly higher abundances. In other respects, independently of zooplankton levels, in those treatments in which phosphorus was added (+P and +NP) they showed both abundances and growth rates significantly higher compared either with those supplied with nitrogen only (+N) or controls. A similar outcome resulting from the manipulation of zooplankton densities was observed for the autotrophic picoprokaryotes and picoeukaryotes (APC and APE respectively). Thus, the presence of zooplankton produced higher numbers of both APC and APE despite of nutrient fertilization. Anyhow, for APC in particular, the simultaneous addition of phosphorus and nitrogen (+NP) yielded the greatest increases, even in the treatments without zooplankton. Contrarily, only in the treatment supplemented merely with phosphorus were observed significant differences of APE abundances between -GRAZ and +GRAZ treatments.

Marked changes were also observed for nanoplankters depending of the presence of zooplankton. Both in controls and in treatments supplemented with nitrogen (+N and +NP) the densities of heterotrophic nanoflagellates (HNF) were slightly higher, although not significantly, when copepods were removed. By contrast, these increases were significantly higher when phosphorus was added

solely, which furthermore coincided with the unique case in which positive growth rates were observed for HNF. Neither with the absence nor with the presence of copepods were observed significant differences for the abundances of phototrophic nanoflagellates (PNF). A noticeable but not significant increase occurred only in the treatment supplemented with both nutrients when zooplankton was removed. On the other hand, a remarkable outcome was observed for ciliates protozoa, which showed significant increases of numbers and growth rates when copepods were absent, with the exception of the treatment supplemented only with nitrogen.

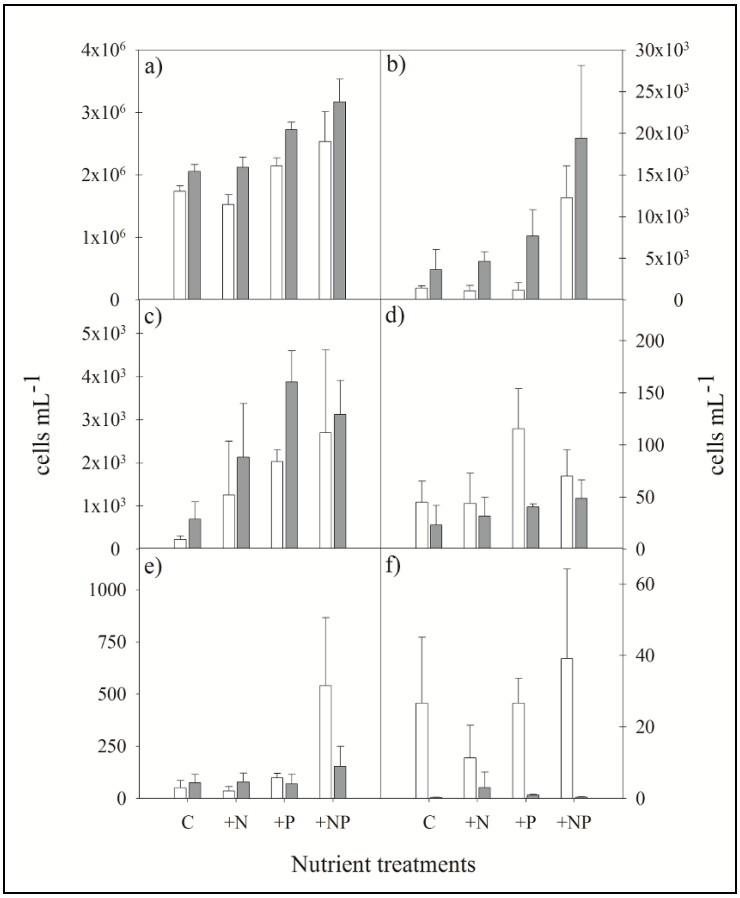

Figure 6.6. *Mean abundances of a) heterotrophic picoplankton (HPP); b)autotrophic picocyanobacteria (APC), c) autotrophic picoeukaryotes (APE); d)heterotrophic nanoflagellates (HNF); e) phototrophic nanoflagellates (PNF) and f) Ciliates at the final of experiment measured in the different enrichment treatments without (white) and with (grey) zooplankton. Error bars indicate the standard deviation.*

6.3.3.3. Changes in phytoplankton community structure

Remarkable changes occurred at the final of bioassay for the total phytoplankton biomass, estimated as chlorophyll-*a* (Chl-*a*) concentration, depending of experimental conditions (Fig. 6.8, and tables 6.4 and 6.5). The initial phytoplankton biomass averaged 0.65 and 0.25 µg Chl-*a* L^{-1} in the treatments without and with zooplankton respectively. At this time, the diatoms and chlorophytes, with a lesser domain of unicellular picocyanobacteria and small nanoflagellates, mainly composed the phytoplankton assemblage. Only small pennate forms with an average cell length of 26.15 µm (±23.37 SD) composed the diatoms subset. The dominant genera were *Fragilaria* (20 µm), *Achnantes* (7 µm), *Diploneis* (15 µm), *Gomphonema* (7 µm), *Nitzschia* (35 µm) and *Stauroneis* (12 µm). Among chlorophytes it was detectable the presence of picoflagellates and larger species such as *Ankistrodesmus antarcticus* (30 µm) and *Cosmarium* spp. (80 µm). Despite of the differences in the Chl-*a* concentrations observed at the commencement of the experiment due to the experimental filtration, a significant net increase occurred at the final of experiment in all treatments in which zooplankton densities were augmented, including controls. These treatments did not show, however, significant differences between them. By contrast, a decrease occurred in all carboys without copepods, except in +NP treatment. This later was furthermore the only treatment showing slight positive net increases.

With regards to the phytoplankton community composition, the effect of zooplankton was stronger compared to the addition of nutrients. The presence of zooplankton at high abundances favoured the dominance of chlorophytes over diatoms and/or chrysophytes as inferred from the higher relative content of lutein compared to fucoxanthin (Fig. 6.9). On the contrary, the removal of zooplankton yielded higher abundances of diatoms and/or chrysophytes and lower of chlorophytes; however, these differences were not significant due to the high dispersion of replicates. Even though, significant differences emerge if the combined lutein to fucoxanthin ratio are considered (Fig 6.9), which clearly shows the higher dominance of chlorophytes (lutein) with respect to diatoms and/or chrysophytes (fucoxanthin) associated to the high zooplankton abundance, principally in those treatments supplemented with phosphorus.

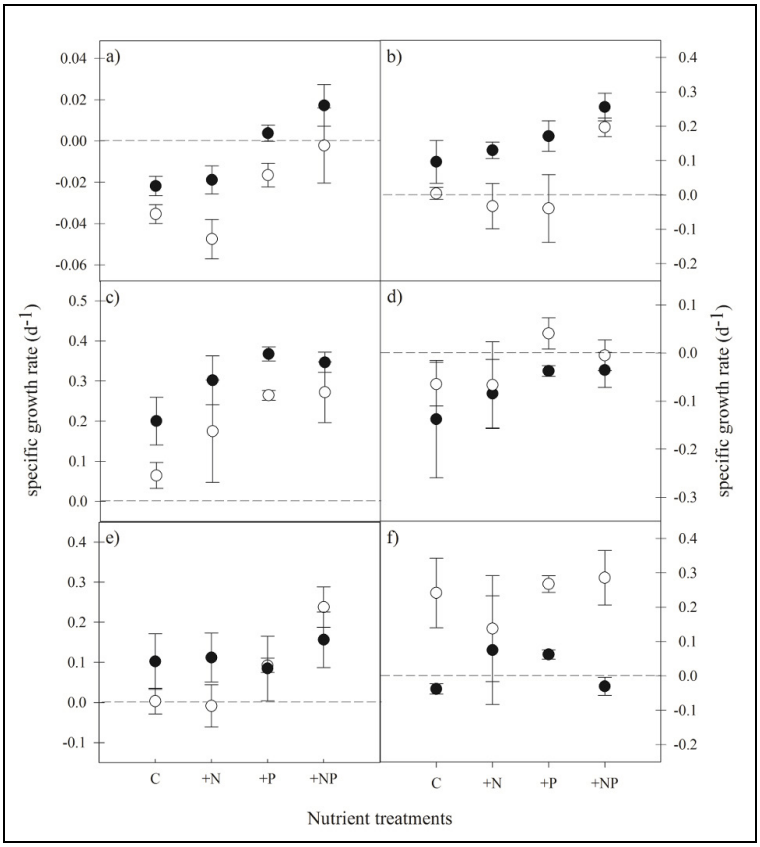

Figure 6.7. *Mean specific growth rates (days^{-1}) of a) heterotrophic picoplankton (HPP); b)autotrophic picocyanobacteria (APC), c) autotrophic picoeukaryotes (APE); d)heterotrophic nanoflagellates (HNF); e) phototrophic nanoflagellates (PNF) and f) Ciliates measured at the final of experiment in the different nutrient treatments without (white) and with (black) zooplankton. Error bars indicate standard deviations. Notice that in some cases errors bars are smaller than the symbols size.*

Table 6.2. *Statistical test for differences in measured specific growth rates of microbial loop components after 11 days of incubation depending on the presence of mesoozooplankton (GRAZ) and fertilization (NUT). The lack of significance is indicated as n.s.*

	GRAZ		NUT		GRAZ*NUT	
Microbial component	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
HPP	31.33	0.00	26.92	0.00	0.77	ns
APC	35.54	0.00	15.37	0.00	2.43	ns
APE	18.42	0.00	11.10	0.00	0.29	ns
HNF	4.12	ns	2.65	ns	0.49	ns
PNF	2.33	ns	8.79	0.00	3.06	ns
Ciliates	33.29	0.00	0.59	ns	2.25	ns

Table 6.3. *Post hoc test (Scheffé) for differences in measured specific growth rates of microbial loop components at 11 days of incubation depending on the presence of mesoozooplankton (GRAZ) and fertilization (NUT). The lack of significance is indicated as n.s.*

Treatment		**Microbial loop members**					
		HPP	**APC**	**APE**	**HNF**	**PNF**	**Ciliates**
-GRAZ	**C *vs* +N**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	C *vs* +P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	C *vs* +NP	0.037	0.032	0.032	n.s.	0.000	n.s.
	+N *vs* +P	0.045	n.s.	n.s.	n.s.	n.s.	n.s.
	+N *vs* +NP	0.006	0.013	0.013	n.s.	0.000	n.s.
	+P *vs* +NP	n.s.	0.011	0.011	n.s.	0.010	n.s.
+GRAZ	**C *vs* +N**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	C *vs* +P	0.012	n.s.	0.014	n.s.	n.s.	n.s.
	C *vs* +NP	0.001	0.017	0.028	n.s.	n.s.	n.s.
	+N *vs* +P	0.024	n.s.	n.s.	n.s.	n.s.	n.s.
	+N *vs* +NP	0.002	0.053	n.s.	n.s.	n.s.	n.s.
	+P *vs* +NP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 6.4. *Statistical test for differences in measured specific growth rates of algal pigments at 11 days of incubation depending on the presence of mesoozooplankton (GRAZ) and fertilization (NUT). ns, not significant.*

	GRAZ		NUT		GRAZ*NUT	
Photosynthetic pigments	**F**	**p-value**	**F**	**p-value**	**F**	**p-value**
Chl-a	201.53	0.000	10.02	0.001	7.62	0.002
Fuco	27.80	0.000	1.86	0.178	3.79	0.031
Lut	125.60	0.000	4.09	0.025	2.45	0.101

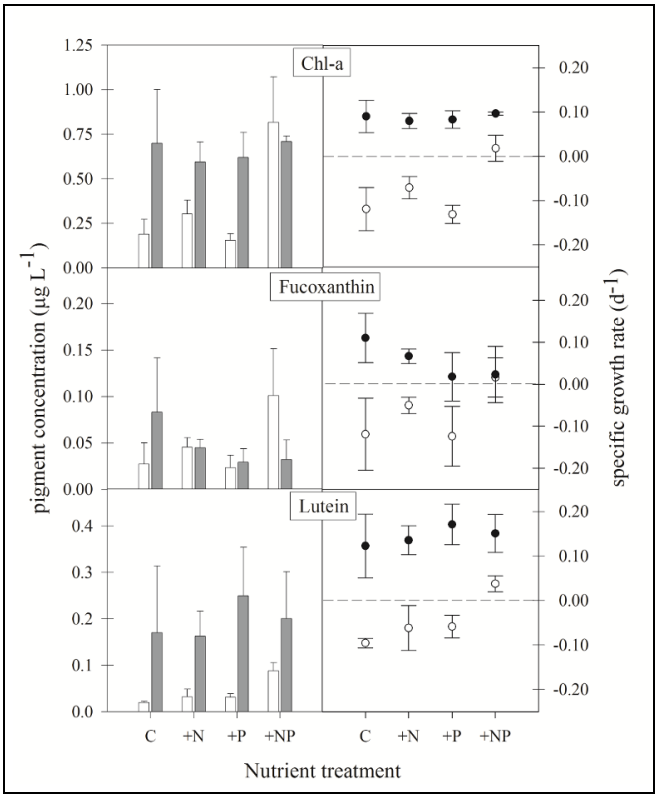

Figure 6.8. *Pigment concentration and specific growth rate (µ: days^{-1}) of Chlorophyll-a and major carotenoid pigments in the different nutrient treatments without (white) and with (grey) zooplankton. Error bars indicate standard deviations in both cases.*

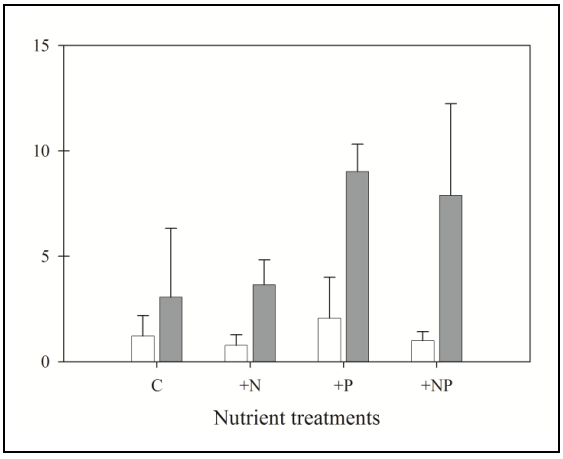

Figure 6.9*. Change on phytoplankton community structure expressed as the quotient between lutein and fucoxanthin in the different nutrient treatments without (white) and with (grey) zooplankton.*

Table 6.5. *Post hoc test (Scheffé) for differences in measured specific growth rates of algal pigments at 11 days of incubation depending on the presence of mesoozooplankton (GRAZ) and fertilization (NUT). ns, not significant.*

Treatment		Photosynthetic pigments		
GRAZ	**NUT**	**Chl-a**	**Fuco**	**Lut**
-GRAZ	**C versus +N**	n.s.	n.s.	n.s.
	C versus +P	n.s.	n.s.	n.s.
	C versus +NP	0.007	n.s.	0.005
	+N versus +P	n.s.	n.s.	n.s.
	+N versus +NP	n.s.	n.s.	0.024
	+P versus +NP	0.004	n.s.	0.029
+GRAZ	**C versus +N**	n.s.	n.s.	n.s.
	C versus +P	n.s.	n.s.	n.s.
	C versus +NP	n.s.	n.s.	n.s.
	+N versus +P	n.s.	n.s.	n.s.
	+N versus +NP	n.s.	n.s.	n.s.
	+P versus +NP	n.s.	n.s.	n.s.

6.3.4. Experiment II

6.3.4.1. Copepods community structure during the experiment

The experimental manipulation was successful in creating a gradient of *Boeckella poppei* abundances, which ranged at the final of experiment from 0 to 136 ind l^{-1} and 0 to 159 ind l^{-1} in supplemented (+NPSi) and non-supplemented (-NPSi) carboys respectively. These numbers represent a density gradient covering two orders of magnitude, which is in the range of natural abundances observed in Lake Limnopolar. With regards to the population size structure (Fig. 6.10), it differed rather from the observed at the experiment I. Here, no nauplii stages were neither observed and the immature copepods were slightly more represented. Even though there not were significant differences among treatments with regards the size distribution of copepods, in some treatments receiving nutrients it seemed to be more slightly skewed towards mature stages, particularly in the enclosures bearing the higher copepods densities (8x-32x).

6.3.4.2. Relation between nutrients and copepods densities

Experimental enrichment resulted in an increase of inorganic nitrogen and phosphorus around 75-folds compared to the ambient concentrations. The different dissolved forms of nitrogen varied in any case depending also on copepods densities (Fig. 6.11). The dissolved organic nitrogen (DON) in the non-fertilized treatments when experiment concluded was always the main form, although they were only significantly higher in the treatment without copepods (0x). With regards to the combined forms, the concentrations of nitrate ($N0_3^-$) in the non-fertilized treatments were consistently around 0.22-0.78 µM, whereas concentrations of ammonium (NH_4^+) increased gradually from 0.6 to 3.5 µM as copepods density increased. As a result, and due to the minor variation of phosphorus concentrations, an increase of the DIN/SRP molar ratios was detected at the higher copepods densities, thus prevailing in this case a moderate phosphorus limitation (DIN/SRP=45). The later contrasted with the observed in those treatments wherein metazoan were totally removed (<50 µM and 0x), which showed DIN/SRP molar ratios consistently below 15. The enriched enclosures displayed, by contrast, an inverse trend. In this case, the inorganic fraction overcame at the final of experiment, except in treatments with higher copepods densities. This occurred because of a gradual augment of DON concentrations as copepods densities augmented. A substantial shift in the distribution of dissolved forms of phosphorus was observed also. As expected, phosphorus prevailed as inorganic, mainly in the fertilized treatments. Even so, the dissolved organic phosphorus (DOP) in the non-supplemented enclosures was in average 38% (±12SD) of the total dissolved phosphorus, and even 50% at the highest density of copepods.

6.3.4.3. Changes in the structure of microbial community

Similarly to the observed in the previous experiment, the presence of metazoan zooplankton impacted strongly the microbial loop (Fig. 6.12). Regardless of nutrient additions, an increase in copepods densities provoked increases of bacterial numbers, following roughly a power function reponse (Fig. 6.12a). Concerning the frequency of bacterial dividing cells (FDC; Fig. 6.13), their mean values were always lower than 8% in all treatments. As a rule, in those treatments in which nutrients were added the FDC attained the higher values compared to the treatments none supplied. These differences were, however, only significant ($p>0.05$) when copepods were in very low numbers (0-1 copepods L^{-1}). Contrariwise, only at higher copepods densities (160 copepods L^{-1}) the FDC values were higher but not

significant in the treatments without nutrients supplementation compared to the others. On the other hand, neither discernable trend for this variable was observed in relation to copepods densities.

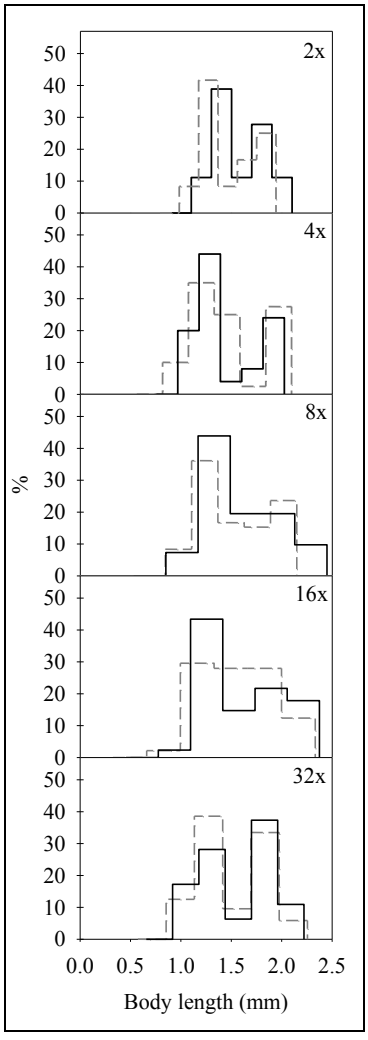

Figure 6.10. *Size structure of Bockella poppei population at different experimental conditions at the final of experiment. Solid and dashed lines indicate –NPSi and +NPSi treatments respectively.*

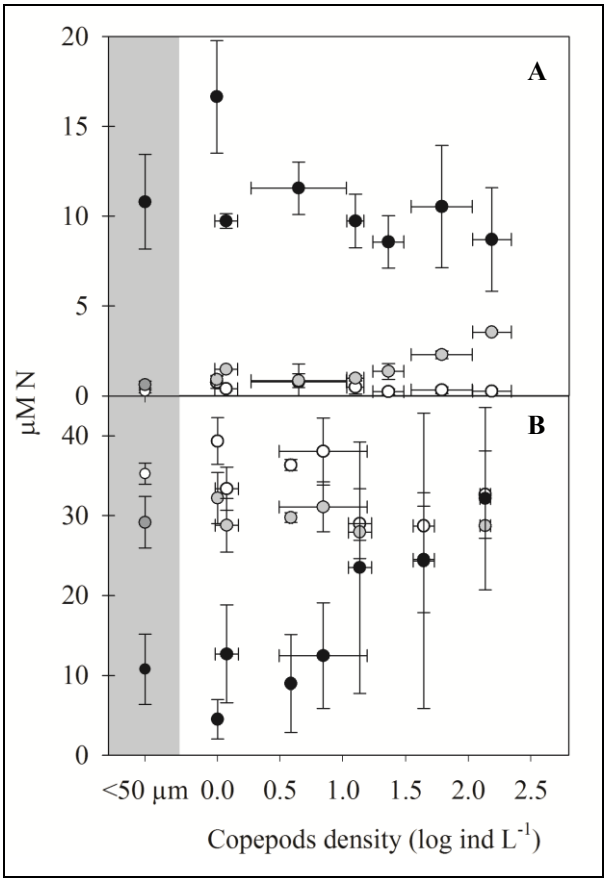

Figure 6.11. *Nitrogen dissolved forms in different treatments at the finish of Bioassay II as a function of copepods density. A) without nutrients addition (-NPSi), B) without nutrients addition (+NPSi). Circles are nitrate (white), ammonium (grey) and dissolved organic nitrogen (black). Note the differences between scales of two plots. Error bars indicates standard deviation of mean.*

In opposition to bacteria, autotrophic picocyanobacteria (APC) were more dependent of nutrients (Fig 6.12b). Thus, although a positive effect was observed in general in response to copepods densities, it was damped in the unfertilized treatments. Differently to bacteria and regardless of nutrients additions, the increases of APC numbers saturated at copepods densities of around 50 ind L^{-1}. In a similar manner, the abundances of autotrophic picoeukaryotes (APE) increased in parallel to copepods densities, being normally higher in the fertilized enclosures (Fig 6.12c). Concerning nanoplankters, the abundances of heterotrophic forms (HNF) were consistently low and neither clear pattern was observed depending of any

experimental condition (Fig. 6.12d). In relation to plastidic nanoflagellates (PNF), despite to the scatter of some replicates, a consistent and steep reduction of numbers occurred as copepods density increased (Fig. 6.12e). Remarkably, their abundances were consistently lower at all copepods densities in the treatments supplemented with nutrients compared to those unfertilized. The ciliates underwent also a strong reduction in numbers (Fig 6.12f), but differently to PNF, they showed an analogous response in both supplemented and none-supplemented treatments.

Regardless of the abrupt depletion of nanoplankters, some linear correlations arose between them and picoplankters (Table 6.6). In the unfertilized treatments, HPP correlated positively with HNF and negatively with PNF and ciliates. APC showed similar outcomes although the negative correlation with ciliates was not significant. When nutrients were added, both HPP and APC also correlated positively and negatively with HNF and ciliates respectively but no with PNF. By contrast, neither nanoplankter showed a significant correlation with APE. Among the picoplankters, HPP and APC only correlated both positively when nutrients were added. By contrast, a positive correlation between APE and APC only occurred in the unfertilized treatments.

In terms of biovolume the previous outcomes resulted in a consistent and directional change of the microbial assemblage structure as the densities of copepods increased (Fig. 6.14). Therefore, nano-sized protist dominated at low copepods densities, whereas pico-sized organisms improved their relative dominance as copepods abundances increased, particularly in those treatments receiving nutrients. The relative abundances of APC ranged 5-45% and 10-62% in –NPSi and +NPSi sets respectively, with great abundances occurring at higher copepods densities. Likewise, APE increased relatively with the augment of copepods, mainly in the unfertilized treatments. Their relative abundances ranged then 2-22% and 7-19% in –NPSi and +NPSi series respectively. On the other hand, the relative abundances of HNF were generally below 2%, although a slight augment (~10%) was observed in the absence of experimental fertilization at the highest copepods density. By contrast, the PNF dominated in the non-supplemented treatments, with higher relative abundances occurring at the treatment 1x. The ciliates were more abundant in nutrient-rich carboys, reaching relative abundances up that 60% of total biomass in the total absence of copepods (i.e. <50 µm and 0x treatments).

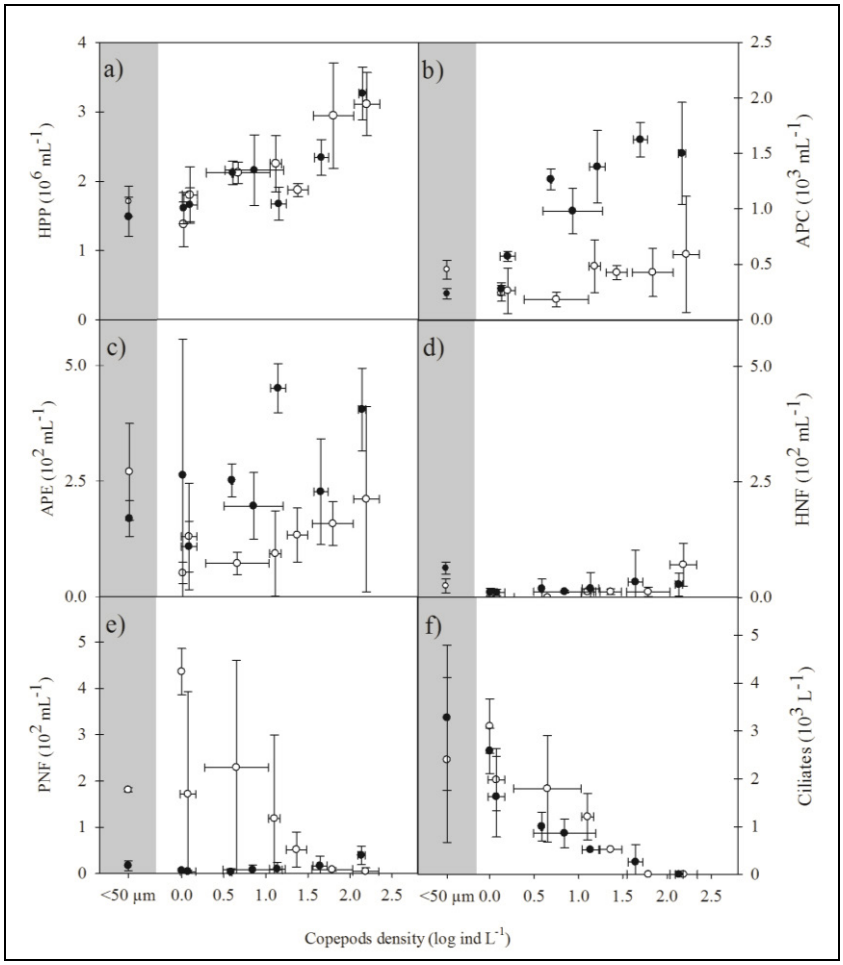

Figure 6.12. *Mean abundances (±SE) of microbial loop components as a function of natural logarithm of copepods density.* ***Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF) and ciliates.*** *The unfertilized (-NPSi) and fertilized (+NPSi) treatments are indicated by white and black circles respectively.*

Table 6.6. *Spearman correlations for the relationship between densities of different microbial members in function of copepods densities. Symbols * and ** indicate significant differences between the mat layers with p>0.05 and p>0.01 respectively.*

Nutrient treatment		APC	APE	HNF	PNF	Ciliates
-NPSi	HPP	0.548	0.31	0.708*	-0.810**	-0.922**
	APC		0.690*	0.878**	-0.714*	-0.515
	APF			0.512	-0.619	-0.419
	HNF				-0.830**	-0.749*
	PNF					0.922**
+NPSi	HPP	0.881**	0.333	0.667*	0.286	-0.929**
	APC		0.500	0.833**	0.286	-0.952**
	APF			0.524	0.214	-0.524
	HNF				0.595	-0.762*
	PNF					-0.405

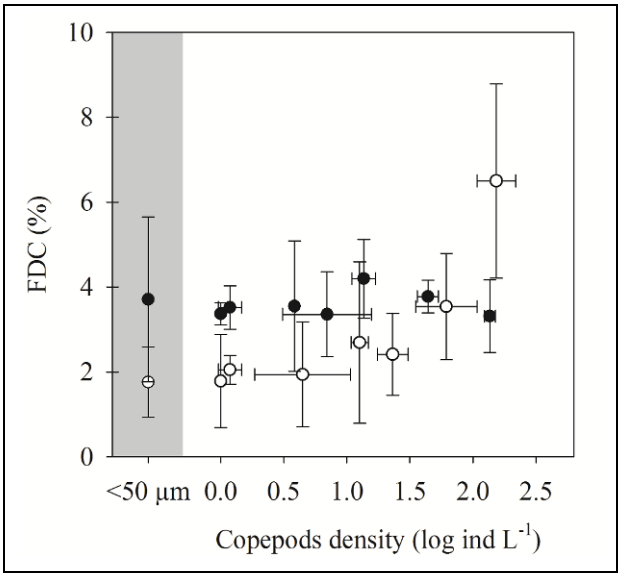

Figure 6.13. *Mean abundances (±SE) of frequency of bacterial dividing cells (FDC) components as a function of natural logarithm of copepods density. Unfertilized (-NPSi) and fertilized (+NPSi) treatments are indicated by white and black symbols respectively.*

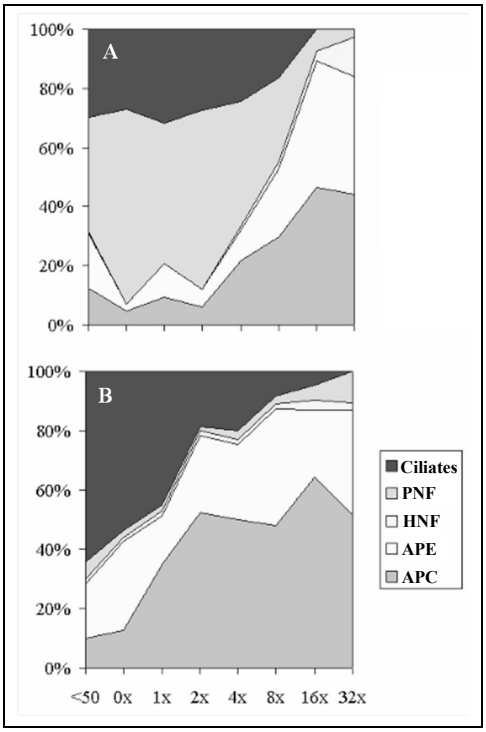

Figure 6.14. *Microbial community structure as function of zooplankton densities and nutrient enrichment. A) Unfertilized treatment (-NPSi, B) Fertilized treatment (+NPSi). Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF) and ciliates.*

6.3.4.4. Changes in bacterial community structure

In the figure 6.15 are shown the log normal distributions of cell size and aspect ratio of bacterial population resulted from the different experimental conditions. Average values for both parameters in each treatment are shown in figure 6.16. More than 90% of bacterial population had a volume smaller than 1 μm^3 in all treatments. Cocci and rod-shaped cells mainly composed the assemblages, whereas the filamentous cells never exceeded the 2%. With the complete absence of copepods (<50 μm), bacterial volume was in average significantly higher when nutrients were not added. Even though, a positive selection of small and large cells was noticed in this case, which produced a flattering in the distribution curve of bacterial size (Fig. 6.15). Despite of the nutrient supplementation, the average size of bacterial cell declined more markedly in the range of copepods densities between 0 to 14

copepods L^{-1}. This drop saturated, however, in the enclosures affiliated with higher copepods densities (23-160 copepods L^{-1}).

Bacterial shape was typified as the cell aspect ratio; that is, the quotient between the length and width dimensions of the cell. The differences observed were apparently modulated overall by nutrient availability instead of copepods densities. When copepods were present (i.e., all treatments except <50 µm and 0x), the aspect ratio was in average regularly greater in the unfertilized enclosures, still, only the treatments 1x, 2x and 16x displayed significant differences. It was provoked by a higher occurrence of rod shaped cells in the assemblages. By contrast, regardless of nutrient fertilization, the cell aspect ratio remained in average nearly constant despite the density of copepods. Additionally, it was observed at times inclusions inside the cells of these bacilli. These granules distributed homogenously inside the cells (Fig. 6.17a) and were easily recognizable by an irregular DAPI stain. As shown in figure 6.17b, the positive stain with calcofluor indicated they were composed of carbohydrates.

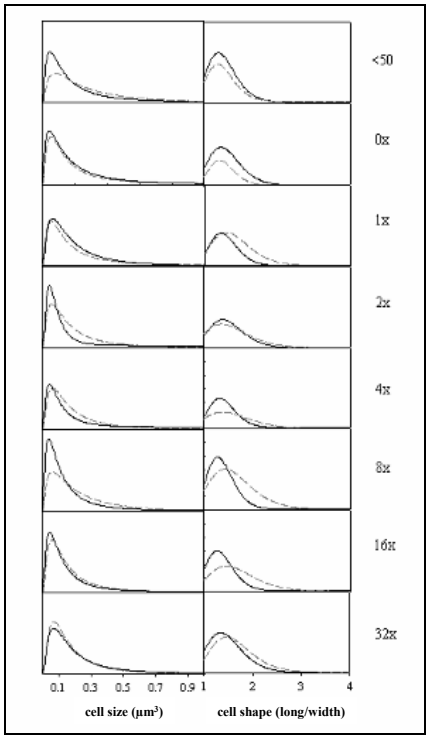

Figure 6.15. *Distribution of the cell size and aspect ratio in bacterial assemblage at different copepods densities for-NPSi (dashed line) and +NPSi (solid line) treatments. The curves represent the fitting of data to a log normal distribution.*

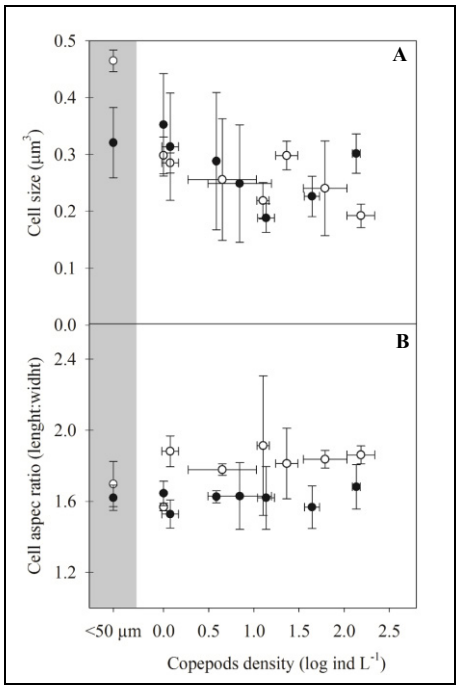

Figure 6.16. *Mean values of cell volume (A) and aspect ratio (B) of bacterial population at the final of experiment depending of nutrient and copepods densities treatments. Unfertilized (-NPSi) and fertilized (+NPSi) treatments are indicated by white and black symbols respectively.*

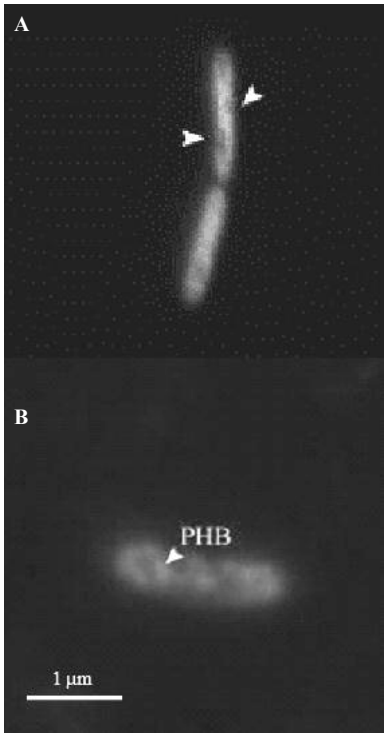

Figure 6.17*. Microphotograhs of bacteria taken at 1250x magnification in an epifluorescence microscope. A) DAPI stained cell showing heterogeneous staining (picture converted to grey scale). B) Calcoflour white stained cell showing carbohydrate inclusions.*

6.3.5. Experiment III

The time-course dynamics of microbial loop members are shown in figures 6.18. In the figure 6.19 is represented in box-wisker plots the spread of data. When experiment started, the initial concentrations of heterotrophic (HPP) and autotrophic picocyanobacteria (APC) averaged $1.02x10^6$ and 621 cells mL^{-1} respectively, showing the experimental handling to have a minor effect on treatments. Small flagellates numerically dominated the nanoplankton assemblage when experiment started, with total abundances averaging in this case 177 cells mL^{-1}. Between them, the heterotrophic forms (HNF) dominated, being around 4-fold more abundant. Plastidic flagellates (PNF) were mainly composed by picoplanktonic and nanoplankters forms such as *Pseudokephyrion*. The initial average numbers of ciliates were around 5 ind mL^{-1}.

The HPP showed important variations between treatments. After 12 hours, numbers increased significantly in the unfiltered carboys compared with the two pre-filtered treatments (<150 and <50 µm). These differences were fading out until 60 h, after which numbers in <150 µm and mainly in <50 µm enclosures fallen once more. At the final of experiment (156 h), significant higher abundances were observed again in the unfiltered treatment. As observed for HPP, the growth rates of APC were significantly stimulated in the unfiltered treatment at 12 h and also in treatment filtered by 150 µm at 24 h compared to the <50 µm enclosures. A general drop in APC abundances occurred, however, from 38-60 h, principally in the two treatments pre-filtered, especially in the <50 µm. This coincided in time with the occurrence of an increase of autotrophic picoeukaryotes (APE) in all treatments.

Differently to picoplankters, nanoplanktonic protists were enhanced by filtration. The HNF showed notable and significant increases at 12 h in both treatments filtered by 150 and 50 µm. Coinciding with the period at which picoplankton populations decreased, it was observed also a drop of HNF abundances, although they maintained stable numbers of around $2.5x10^2$ mL^{-1} in the treatment filtered by 150 µm. At the final of experiment, great increases although no significant occurred in this treatment compared to the others. The subset of PNF was favored also when grazers were removed but in a lesser extent. Thus, both filtered treatments showed high numbers at 12 h compared to unfiltered enclosures, though, differences were only significant in the treatment filtered by 50 µm. On the contrary, more important increases of PNF compared to HNF were observed in the treatment filtered by 150 µm when enclosures were retrieved at 156 hours, just coinciding with their higher abundances (~7 10^2 mL^{-1}). With relation to ciliates, they showed also important increases at the commencement of experiment. Concretely in the

enclosures pre-filtered by 50 µm they displayed an early phase of exponential growth until 24 h. Subsequently populations collapsed, even though, they generally maintained their higher abundances on the total exclusion of zooplankton (<50 µm). Among the dominant ciliates, *Balanion planctonicum* dropped sharply, whereas *Cyclidium sp.* declined more progressively along time.

In relation to the stability of the time-course dynamics, growth rates of all microbial components varied greatly when metazoan were partially or totally removed (Fig. 6.20 and 6.21), being these differences minor in the case of HPP. Both APC and APE varied more greatly in the treatment pre-filtered by 50 µm. On the other hand, nanoflagellates showed great variations in the treatment pre-filtered by 150 µm, mainly the heterotrophic forms, whereas ciliates varied more markedly in the treatment pre-filtered by 50 µm. Concerning to the co-variation between them (Tables 6.7 and 6.8), both numbers and growth rates of HPP and APP showed a positive relationships among them at all conditions assayed. By contrast, the HPP and APE numbers correlated negatively in the two pre-filtered treatments. In the case of HNF, neither significant correlation was observed between their abundances and the other microbial members, in any case, their growth rates still correlated negatively with HPP in the treatment pre-filtered by 150 µm and positively with APF and APC in the <150 µm and <50 µm treatment respectively. On the other hand, in the total absence of metazoan (<50 µm), the growth rates of PNF varied negatively with HPP and APC. Otherwise, neither significant correlation was observed in those dynamics involving ciliates.

The production and predation losses of protists were estimated by computing in the equations 6.3 and 6.4 the variation observed in their abundances after the first day of the experiment (Fig. 6.22). As a remarkably trend, only the ciliates showed an equilibrium between both processes, which were close to 0.14 ng C mL^{-1} h^{-1}. By contrast, the estimates of predation rates over HNF and PNF exceeded their rates of production. For HNF, production and predation losses were near to 0.04 and 0.06 ng C mL^{-1} h^{-1} respectively. On the other hand, the estimates for PNF were higher, being respectively around 0.06 and 0.12 ng C mL^{-1} h^{-1} respectively.

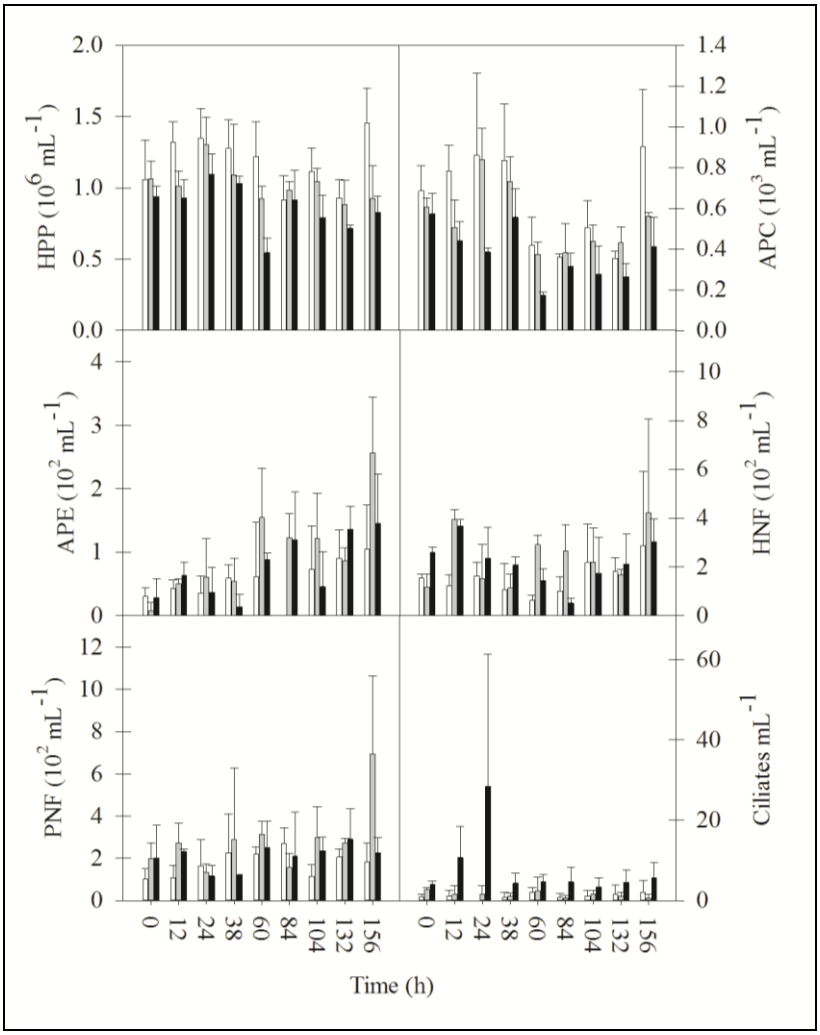

Figure 6.18. *Evolution of abundances of different microbial members during experiment III at the three conditions assayed: without filtration (white bars), filtrated through 150 µm (grey bars), and filtrated by 50 µm (black bars). Acronyms are as follow: Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF).*

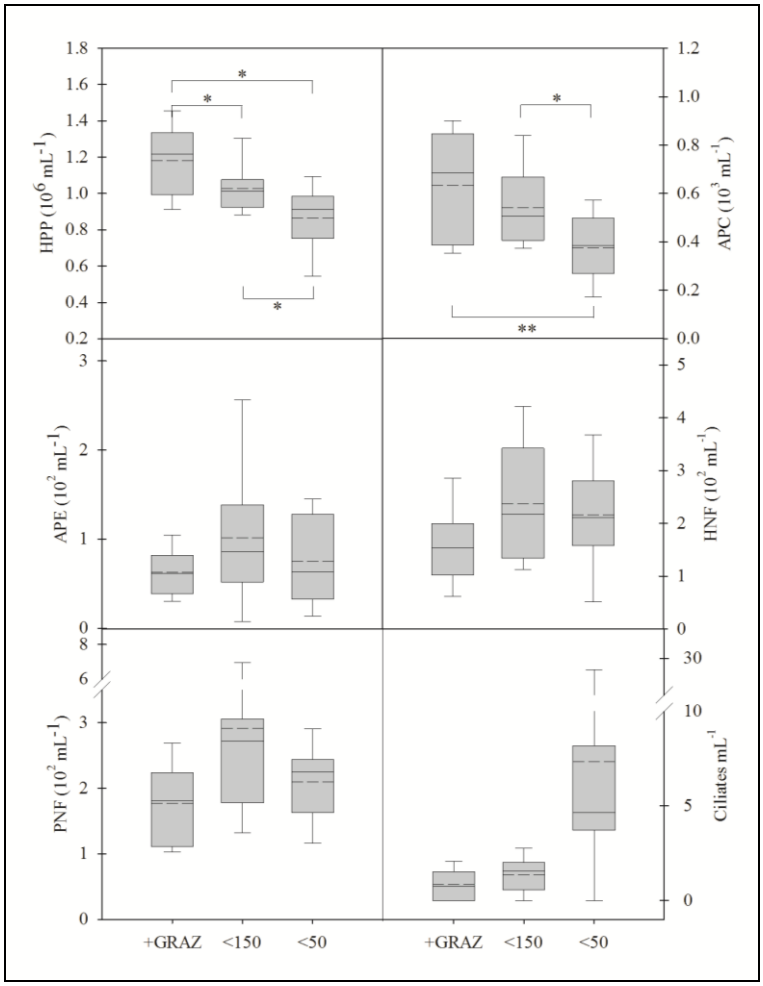

Figure 6.19. *Box plots showing the distribution of mean abundances of different microbial members in each treatment. Both * and ** indicate significant differences at levels 0.05 or 0.01 (1-tailed) respectively. Acronyms are as follow: Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF).*

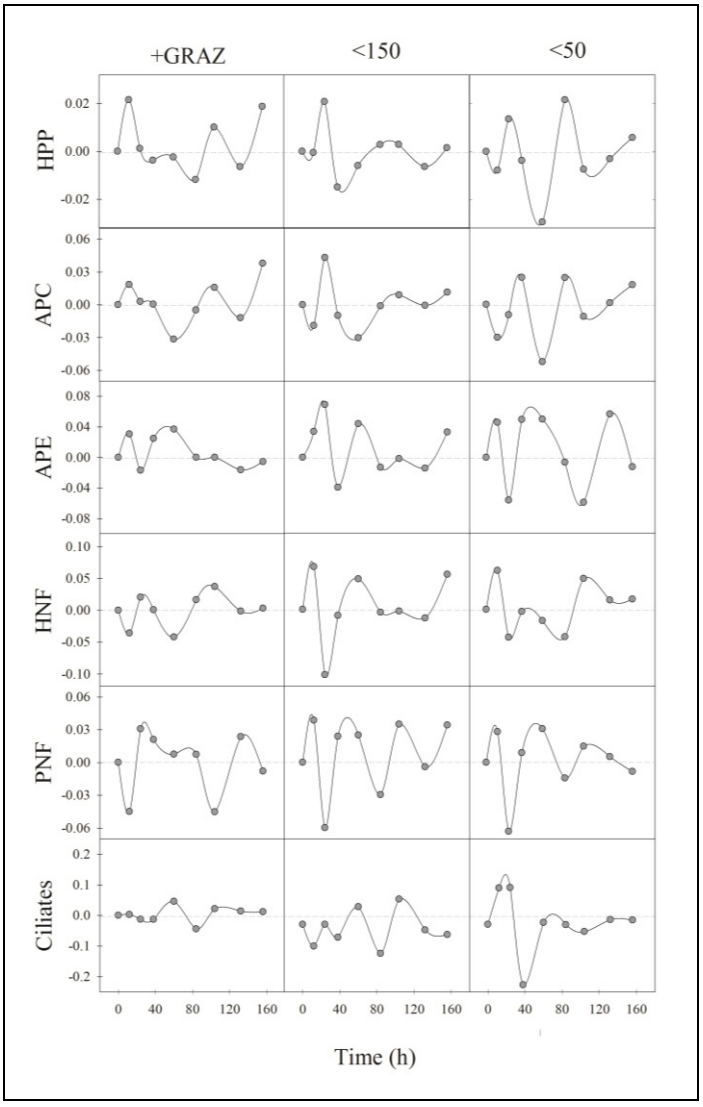

Figure 6.20. *Growth rates of different microbial members during experiment III at the three conditions assayed. Acronyms are as follow: Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF).*

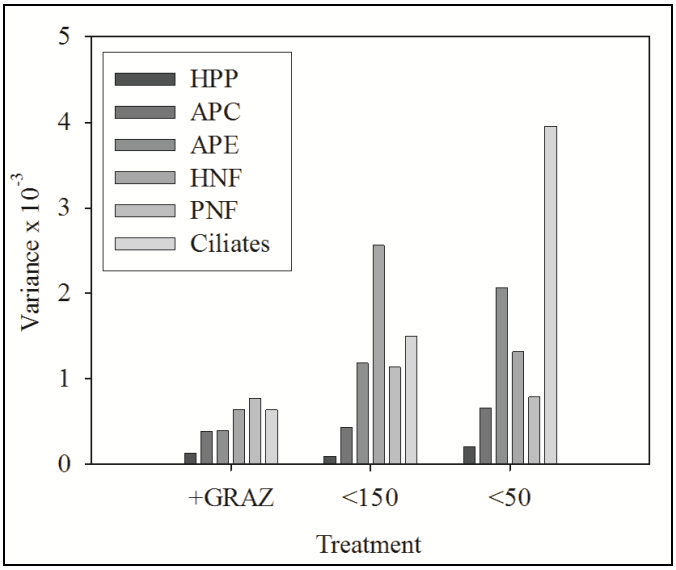

Figure 6.21. *Variance of the growth rates during experiment at different treatments for each microbial member. Acronyms are as follow: Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF).*

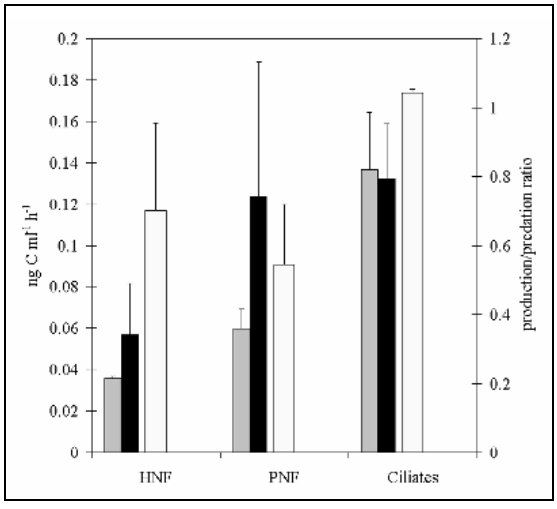

Figure 6.22. *Biomass production (dark grey), biomass losses by predation (black) and the quotient among them (clear grey) for different groups of protists.*

Table 6.7. *Spearman correlations for growth rates between different microbial members at three different conditions assayed. Both * and ** indicate that correlation are significant at levels 0.05 or 0.01 (1-tailed) respectively. Acronyms are as follow: Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF).*

Treatment		APC	APE	HNF	PNF	Ciliates
GRAZ	HPP	0.833**	0.051	0.067	0.600*	0.317
	APC		-0.170	0.417	-0.550	-0.083
	APE			-0.559	-0.373	0.170
	HNF				-0.100	-0.333
	PNF					-0.283
<150	HPP	0.700*	0.450	-0.100	-0.183	0.234
	APC		0.150	-0.350	-0.300	0.293
	APE			0.333	0.100	0.335
	HNF				0.800**	-0.092
	PNF					0.092
<50	HPP	0.633	-0.433	-0.483	-0.983**	0.100
	APC		0.017	-0.233	-0.567	-0.417
	APE			-0.017	0.467	0.050
	HNF				0.500	0.017
	PNF					-0.200

Table 6.8. *Spearman correlations for abundances between different microbial members at three different conditions assayed. Both * and ** indicate that correlation are significant at levels 0.05 or 0.01 (1-tailed) respectively. Acronyms are as follow: Heterotrophic picoplankton (=bacterioplankton) (HPP), Autotrophic picocyanobacteria (APC), autotrophic picoeukaryotes (APE), heterotrophic nanoflagellates (HNF), Plastidic nanoflagellates (PNF).*

Treatment		APC	APE	HNF	PNF	Ciliates
GRAZ	HPP	0.900**	-0.083	0.300	-0.250	0.441
	APC		-0.233	0.333	-0.300	0.254
	APE			0.433	0.417	0.492
	HNF				-0.467	0.051
	PNF					0.199
<150	HPP	0.817**	-0.600*	-0.617*	-0.452	0.385
	APC		-0.550	-0.517	-0.285	0.134
	APE			0.617*	0.544	-0.318
	HNF				0.469	-0.142
	PNF					0.419
<50	HPP	0.783**	-0.683*	0.367	-0.933**	0.400
	APC		-0.550	0.600*	-0.700*	0.183
	APE			-0.017	0.617*	0.033
	HNF				-0.167	0.367
	PNF					-0.417

6.4. Discussion

Our experiments show forceful evidences for the potential existence of a trophic cascade in the microbial pelagic food web of Lake Limnopolar. This is mediated by a strong top-down regulation of protozoa populations by copepods, which indirectly benefits pico-sized organisms (both autotrophic and heterotrophic). This points out how in low productive ecosystems the microbial loop increases its relative importance in relation to the classical herbivore pathways. The grazing activity of ciliates is not scored here because some methodological problems, however, during a field survey performed in Otero Lake (northwest of the Antarctic Peninsula) by MATALONI ET AL. (2002) these authors found, in accordance with us, that nanociliates up to $2x10^3$ μm^3 grazed mostly on pico-sized organisms, while larger ones feed on nanoplankters. Moreover, our results state the non-linear character of these interactions. In the experiment II it is observed, for instance, how copepods match the ciliates and nanoflagellates production even when the former are in low numbers. However, the strong depletion of nanoplankters and the elevated deviation observed in some replicates led to believe that stochastic process may also control these interactions. It is interesting to mention here the outcomes of GISMERVIK (2006), who demonstrated experimentally how small copepods with a filter-feeding behaviour (*Pseudocalanus* sp.) had a great impact on bacteriovorus population (ciliates in that case), leading to the extinction of populations, as they do not decrease their clearance rates at low food concentrations.

The outcomes of the experiments with microspheres bolster the idea that a trophic cascade occurs in the lake and furthermore describe some of its mechanical aspects. The calanoid copepods such as *B.poppei* show generally a feeding mode based on a filtering mechanism. This involves the motion of thoracic appendixes, which propels a flow toward the mouth, where the food is filtered by setules. Accordingly, there is a positive correlation between the prey size and the distance between these setules (HESSEN 1985), which at the end depends of the copepod age. Our results agree with this idea. So, they show red microespheres (diameter = 10 μm) as preferred particle-size ingested by adults, which are near to the size of *Balanion planctonicum* and *Cyclidium* sp., the dominant euplanktonic ciliates occurring in Lake Limnopolar, and also quite similar to most of nanoplanktonic diatoms observed in the lake. By contrast, juvenile stages preferentially choose blue microespheres (diameter= 5 μm), which size is similar to the most abundant nanoflagellates, both heterotrophic and autotrophic. On the other hand, there is a clear unresponsiveness for blue microspheres (diameter= 20 μm) in all the stages, which is in conformity with observations that show these copepods to predate

preferentially on algae with a greatest axial lineal dimension smaller than 25 μm (EDGAR AND GREEN 1994).

The experiment with microspheres does not delimit entirely the size range of particle ingested by the copepodid stages, in any case, they are below the optimal size range considered for small calanoids (14-32 μm ESD; HANSEN ET AL. 1994). Otherwise, the experiment reveals not only qualitative but also quantitative differences between immature and adult stages. Thus, we observe a positive relationship between the body size and the grazing rates, which is in agreement with the observed in previous studies (MORALES ET AL. 1990; BAUTISTA AND HARRIS 1992). In other respects, considering that the detection and capture of particles may depend of active motions, not only size but also the swimming behaviour of protozoa may do them more disposed to be predated. In marine ecosystems, for instance, copepods predated mainly over ciliates compared to flagellates sizing similar, which has been explained in these terms (LEVINSEN ET AL. 2000, YANG ET AL. 2009). In that case, the authors proposed that faster swimming ciliates have higher prey–predator encounter rates, which make them more suitable as prey. Nevertheless, this is an issue that can not be confirmed in our experiments.

Both ciliates and nanoflagellates profited for all copepod stages, however, it seems that a preferential pathway from the former prevails. It has been occasionally observed how the predation on ciliates may exceed the observed for algal populations (MERRELL AND STOECKER 1998; HANSEN 2000), which by the way demonstrates the capability of some crustaceans to feed on more than one trophic level. Considering the values reported for the mandible edge index of *Boeckella* (784±34SD; BALSEIRO ET AL. 2001), which characterize the feeding mode (ITOH 1970), this genera can be classified as omnivore. It can be interesting to contrast here the continental and maritime regions of Antarctica. In a comparison between Antarctic lakes from different locations ROBERTS AND CO-WORKERS (2004) showed how ciliates dominated over heterotrophic nanoflagellates (HNF) in continental lakes in which a top-down grazing control was absent (i.e., Lakes Hoare and Fryxell). This differed with the observed in lakes from the maritime region (i.e., Signy Island Lakes), in which *B.poppei* was present and some top-down grazing control existed. Furthermore, in the latter case HNF dominated. These authors did not establish, however, a direct causality between both trends.

Our outcomes can also be compared with that of TRANVIK AND HANSSON (1997) as these authors demonstrated a similar cascading effect mediated by metazoan at South Georgia Islands. In that case, the grazing exerted by the copepod *Boeckella michaelsenii* on flagellates favoured bacterioplankton abundances,

whereas the bigger *Pseudoboeckella* (=*B.poppei*) had no effect on microbial components because of their preference for higher preys. Our results seems to be more consistent with those of BUTLER ET AL. (2005), who evaluated the clearance rates of *B.poppei* in Sombre Lake (Signy Island), and found that this specie grazed preferentially on the small flagellates dominating in the assemblages, but also on nano-sized ciliates. These discrepancies are maybe due to some phenotypic flexibility of this copepod, in such a way that this copepod regulates its size depending of environmental conditions. If so, this phenotypic variation should be controlled by bottom-up factors, since predation is supposedly excluded due to the lack of fishes.

Interestingly, a size dimorphism of *Pseudoboeckella poppei* has been observed in South Georgia (HESSEN ET AL. 1989), being the large morph recorded in lakes, whereas the small was found in ponds. However, we fail to know if either (of both) were those studied by TRANVIK AND HANSSON. With all, seems evident that there is a lower functional diversity of predators in Byers and Signy Island (i.e., only one copepod and scarce rotifers) compared to South Georgia Islands (i.e., two copepods). We conjecture that this lower diversity could be offset by the ability to obtain nutritional requirements from several food sources (i.e., omnivore feeding), which is known to increase growth efficiencies of copepods (COUCH ET AL. 2001). Thereby, *B.poppei* would obtain additional requirements by eating on ciliates or rotifers rather than profiting solely from an algal diet.

In the experiment I we demonstrate that top-down mechanisms act as a shaping force that produce a redistribution of the relative abundances of algal groups. Thus, the presence of zooplankton favours chlorophytes against diatoms and chrysophytes, as indicated by the ratios of taxa specific carotenoids. It is known that the magnitude of the trophic cascade greatly depends of the edibility of the species implicated (POLIS ET AL. 2000; RONDEL ET AL. 2008). There are probably different mechanisms explaining this issue. On the one hand, it is possible that the large size of desmid green algae (*Cosmarium* spp.) and the special shape of *Ankistrodesmus falcatus* make them inedible for zooplankton. This larger chlorophytes supposedly have lower nutrient uptake efficiencies compared to smaller species, though, they would offset this avoiding grazing. On the other hand, pico-sized chlorophytes also appears to be out of the prey range of adult copepods.

There is a compensatory effect emerging from these trophic interactions. Thus, in the experiment I, all treatments including zooplankton yielded higher Chl-*a* concentrations compared to those in which zooplankton was removed. It seems that a fertilization effect is high enough to overcompensate the grazing caused by

copepods on overall phytoplankton community, since comparable growth in the zooplankton lacking treatments was only achieved when both nitrogen and phosphorus were jointly added. The supply of inorganic nutrients via excretion by zooplankters has been shown to promote short-time fertilization effects (VANNI 2002). Some studies point out furthermore a greater fertilization potential of copepods (VREDE AND VREDE 2005). Also, in experiments contrasting the effects of *Boeckella dilatata* (copepod) and *Ceriodaphnia dubia* (cladocera), results showed as both inorganic nitrogen and phosphorus levels increased significantly when copepods instead of cladocerans were present (BURNS AND SCHALLENBERG 1998). However, the regeneration of phosphorus in our study seems to be minor. Probably it is because *B.poppei* relatively sequesters more phosphorus for growth. In the experiment I, a phosphorus limitation affecting HPP is suggested since comparable growth rates occurs between the controls and the treatments supplied with nitrogen and copepods, which contrasts with the observed in treatments without copepods and fertilized with phosphorus. Indeed, the growth rates of microbial components in this experiment were generally lower in the microcosms supplied only with nitrogen, thus providing more evidences for a phosphorus limitation.

There are evidences in the experiments proving the occurrence of a nitrogen turnover driven by zooplankton. The higher ammonia concentrations in the experiment I are found when copepods are abundant, differing with the total ammonia exhaustion when absent. In this experiment, a fertilization effect on the autotrophic picoplankton (APC) is observed in both phosphorus-amended treatments (P and N+P), where nitrogen supplied by zooplankton can complement the added phosphorus to support the growth of APP. Contrarily, when this nitrogen fertilization does not occur (zooplankton absent), the increase of APC populations was only found when both nutrients were jointly supplemented. In the experiment II a nitrogen regeneration also occurs, both in –NPSi and +NPSi treatments, but interestingly it takes place via NH_4 or dissolved organic nitrogen (DON) respectively. Also in this experiment, the treatments supplemented with nutrients show usually the higher frequencies of bacterial dividing cells (%FDC). However, these differences are only significant when copepods densities are low, whereas when they are in high numbers the %FDC are equal or even higher despite the lack of fertilization. Here it is noteworthy that HPP increases even when protists are practically eradicated by copepods, which would proof this capacity of copepods to drive the nutrient regeneration.

The dissolution of faecal pellets and the “sloppy feeding” phenomenon are considered both sources of DON originating from zooplankters (DODDS 2002,

BERMAN AND BRONK 2003). Either of these mechanisms might explain the DON increases observed in experiment II. Occasionally, it has been suggested that the direct excretion of DON by copepods would be a small flux relative to other pathways (MILLER AND GLIBERT 1998). These authors observed excretion rates ranging from non-detectable levels to 2 ng atom N copepod^{-1} h^{-1}. We cannot accurately estimate the excretion fluxes hourly; anyhow, they should be around 9 ng atom N copepod^{-1} h^{-1}, which would imply a major role in our case of the sloppy feeding phenomenon. This underscores again the importance of the prey edibility since the sloppy feeding is the consequence of an inadequate feeding mode. The release of DOC by "sloppy feeding" increases when the prey size is larger relative to the copepod, which implies an inefficient food transfer (MØLLER AND NIELSEN 2001, MØLLER 2005). The increase of DON observed in the experiment in parallel to the decrease of DIN gives maybe an indication of the conversion of particulate nutrients (biomass) into dissolved nutrients (DOC). This DON comprises a complex pool of peptides, amino-sugars and urea, which should be readily assimilated by heterotrophs. As commented before, it might explain that bacteria always increase in parallel to copepods densities, even when bacteriovorus are virtually absent. We lack data of DOC stoichometry, however, it is expected a relatively low C:N ratio due to this organic nitrogen turnover, which might enhance the bacterial growth (KROER 1993). A close relationship between the occurrence of zooplankton and the release of dissolved free amino acids has been experimentally demonstrated in advance (RIEMANN ET AL. 1986), though, the factors controlling bacterial production in that case were not completely explained by this process.

There is also a trade-off between the availability of nutrients and the grazing pressure suggested in the experiment III, although affecting protists. In this experiment, all flagellates attain higher densities when metazoan are partially removed (< 150 µm), but no when they are totally excluded (<50 µm). In the former, the predation over them is supposedly relaxed compared to control treatments, but higher compared to the treatment filtered by 50 µm. It is possible that smaller metazoan passing through the 150 µm filters (i.e., nauplii and/or rotifers) induce still some nutrient regeneration, which should be totally impeded in the enclosures filtered by 50 µm. It is known, in this sense, that flagellates are able to directly incorporate dissolved macromolecules besides to particulate carbon (CHRISTOFFERSEN ET AL. 1996). Other idea is that some flagellates profit as food for ciliates, particularly the smaller ones, as observed in other Antarctic lakes (MATALONI ET AL. 2000). If so, the treatment filtered by 150 µm offers the better balance between the metazoan and protozoan grazing over these small flagellates. This is strongly suggested by the fact that the predation rates over HNF and PNF in

the experiment III exceeds in both cases their rates of production (Fig. 5.22). This negative balance only can be explained if predation over nanoflagellates is not totally excluded in the treatment filtered by 50 µm because the presence of ciliates. In other respects, the close balance between the rates of ciliates production and predation losses demonstrates that they are an important food source for metazoan, which can explain the low standing stocks of ciliates regularly observed in Lake Limnopolar. The highest abundances of ciliates observed here, which occur when metazoan are totally removed, points out this idea. However, it seems that they are not able to sustain stable populations along time. It appears that without an external input of nutrients, or without a rapid nutrient turnover, the system became unsustainable after a short period. This supports the idea that copepods are required to maintain this food web structure, both controlling the trophic cascade and recycling nutrients. The drop observed in the picoplankters abundances from 60 h to 132 h could be explained in these terms, as it seems to be caused by an overexploitation of resources because this inefficient nutrient turnover.

In addition to metazoan, bacteriovorus also might facilitate the turnover of nutrients in the lake. As the excretion rates decline with the body mass, the mass-specific rates of these bacteriovorus are expected to be greater. The nutrients turnover driven by bacteriovorus is considered an important mechanism enhancing bacterial production (SHERR AND SHERR 2002; HAHN AND HÖFLE 2001 and articles cited therein). This might explain the elevated concentrations of DON observed in experiment II when both copepods and nutrients were not added, and just coinciding with the higher abundances of protists. The liberation of organic phosphorus by these bacteriovorus is also possible as observed by ANDERSEN AND CO-WORKERS (1986), which mainly occurs if they feed on bacteria in preference to algae. Nonetheless, these authors pointed out that this phenomenon is exceedingly variable and unpredictable, which further agrees with our observations.

In the experiment II, the grazing exerted by protists involves a shift in the bacterial community size structure. Nanoflagellates are able to discriminate between bacterial cells differing in volume (SHERR AND SHERR 1991). Our experiments shows particles sizing 0.5 µm instead of 1 µm as the preferred by bacteriovorus. The agreement between our findings and those of SHERR AND SHERR reinforces the idea that consumption of <1 µm sized bacterial cells are mainly carried out by small flagellates (< 5 µm), whereas the preys greater than 1 µm are more efficiently ingested by flagellates sizing 5–20 µm. Besides this, interesting differences are observed in the experiment depending of copepods abundances. Thus, from 0 to around 15 copepods L^{-1}, the bacteria cell size show in average a consistent drop,

whereas at higher copepods abundances (23-160 copepods L^{-1}) this tendency appears to saturate. Not only the cell size but also bacterial abundances evolve differently in these two scenarios. Hence, bacterial numbers increase in parallel to copepods at the first range of densities (0-15 copepods L^{-1}), but they increase even more steeply from abundances of 23 copepods L^{-1} henceforth. Therefore, at copepods abundances lower than 15 individuals L^{-1}, the bacterial population seems to be mainly regulated by the trophic cascade. However, with an extra supply of DOC, bacteria out compete from algae for inorganic nutrients and, in addition, are totally liberated from protozoa grazing. At these circumstances, they achieve their highest densities and, as well, grow in a more edible size range. A clear sign of the effect of bacterivory is observed in the treatment pre-filtered by 50 μm and no supplemented with nutrients. In that case, the curve of bacterial size distribution flattens because of a bi-directional increase of the larger and smaller cells. More probably, both are too small and large respectively to be efficiently grazed by protozoan, which agree with the often observed (GÜDE 1989; PERNTHALER ET AL. 1996; HAHN AND HÖFLE 2001). Consequently, grazing-resistant morphotypes are enhanced in the bacterial assemblage when protozoan are present. By contrast, the assemblage grows in a more edible size range if bacteriovorus are suppressed by copepods.

Bacteria are able to modify morphology depending of nutrients availability (SAMUELSSON ET AL. 2002). In the experiment II it is observed how bacterial cells elongate when they undergo nutrients deficiency (not fertilized carboys). By contrast, when competition is relaxed by the addition of nutrients, the cell shape is in average significantly shorter. This might be related with the occurrence of polysaccharide inclusions inside the cells (Fig. 6.17). A higher rate of larger cells under phosphorus limited conditions has been reported by MATZ AND JÜRGENS (2003), and seems a general strategy observed in pelagic osmotrophs (THINGSTAD ET AL. 2005). This involves the accumulation of carbon-rich storage polymers as glycogen and poly-b-hydroxybutyrate during nutrient starvation. Supposedly, this is a competitive advantage since phosphorus content relative to carbon is lower in these compounds. In an experiment carry out by LØVDAL ET AL. (2007) these authors proposed that by increasing cell size without an expense of limited nutrients (e.g., using organic carbon) bacteria maximize the uptake of scarce nutrients as phosphorus. This occurs because the diffusive transport of nutrients through the membrane depends of the cell size, in such a way that it increases as the cell surface/volume quota increases. As note LØVDAL and co-workers, any strategy to increase size without thereby increasing proportionally the cellular requirement of the limiting nutrient will give a competitive advantage. In our case, it seems that this

carbon accumulation translates in a cellular elongation, which could be the result of a trade-off between top-down and bottom-up forces.

To some extent, the observed in the isotopic fractionation agrees with the experimental outcomes. The isotopic fractionation suggests for instance that *B.poppei* interact weakly with the benthos and, by contrast, exploits mainly pelagic resources (from pico and/or nano-sized fractions). We are at this point in agreement with BUTLER ET AL. (2005), who refuses that *B.poppei* is exclusively a benthic browser. In any case, *B.poppei* might change its feeding behaviour depending of the environmental conditions. Some authors argue the importance of benthic resources as part of the pelagic zooplankton diet in lakes from high-latitudes (HANSSON AND TRANVIK 2003, RAUTIO AND VINCENT 2006). This idea relies in the simultaneous occurrence, as in Lake Limnopolar, of biomass-rich stocks of zooplankton and low pelagic production, which led to think that carbon requirements should be complemented with the benthic flora (RAUTIO AND VINCENT 2006). On the other hand, the results clearly indicates that *Branchinecta gaini* profits from benthic resources and sediment detritus, which agrees with the observed in experiments carried out in the two polar regions (PAGGI 1996, BERTILSSON ET AL. 2003). This implies a niche separation between both crustaceans, thus facilitating their coexistence. Concerning to other compartments, the delta values of sestonic carbon probably integrate different functional organisms and therefore interpretations should be made with caution. Nevertheless, it seems that highly depleted values of seston coming from the catchment, which is similar to that of mosses, originate from autotrophic biomass. Additionally, the ^{13}C enrichment of lake's sediments might respond to methanogenic activity, which is an anaerobic respiration that produces ^{13}C-rich CO_2 and ^{13}C-poor CH_4 (GU ET AL. 2004, KANKAALA ET AL. 2006).

In summary, we have evaluated the role of some abiotic and biotic forces as drivers of plankton dynamics in Lake Limnopolar. We have manipulated the pelagic food web by altering both predation and resource levels. The removal of top predators cascade down until lower trophic levels, nonetheless, there is also a resource regulation. Thus, we find that zooplankton importantly affects the accessibility of nutrients by participating in its turnover. The role of this nutrient recycling must be significant in this region due to the general oligotrophic conditions. Consequently, our results demonstrate the existence of a trophic structure more complex than the originally envisaged for these types of lakes, thus indicating that an efficient top-down control of microbial loop populations may exist. This is a good example of how biotic interactions can play a key role on the food web configuration despite of the strong physical control, at least when physical

stressors are temporarily relaxed. This results of interest in a scenario of a climatic change, since warming is likely to produce an intensification of the biotic interactions, energy flow and biogeochemical cycles (DORAN ET AL. 2002).

7. Structural and functional study of microbial mats and their relation with carbon and nitrogen cycles

7.1. Introduction

Microbial mats are macroscopic assemblages of microorganisms that integrate a certain metabolic diversity. They thrive in a wide range of habitats including cold extreme environments (VINCENT 2000a). The structure of microbial mats offers a certain degree of homeostasis against environmental instability, a distinctive trend compared to planktonic life forms that explains their relative importance in extreme environments. Certain structural and functional properties of these communities are involved in strategies for coping with environmental stresses such as UV light damage (GARCIA-PICHEL AND CASTENHOLZ 1991; QUESADA ET AL. 1995), desiccation (HOAGLAND ET AL. 1993; MCKNIGHT 1999; POTTS 1994, 2001) or nutrient starvation (WOLFAARDT ET AL. 1998).

Cyanobacteria and diatoms are the major photosynthetic constituents of these microbial mat communities, which also contain heterotrophic organisms such as bacteria, protozoa, nematode and tardigrades. The species composition determines the structure and colour of the microbial mats (STAL 2000). A regular feature of these communities, as described for cyanobacterial mats from the McMurdo Ice Shelf (DE LOS RÍOS ET AL. 2004), is the occurrence of coloured layers in which different species are distributed. In general, the photosynthetic microbial mats develop at the water-sediment interface in environments shallow enough to allow for light penetration. Steep light gradients are created inside the mat due to the strong absorption of incident radiation. The pigment composition, which covers a wide spectrum from chlorophylls to carotenoids, can vary in relative concentration along this vertical gradient of light.

Both cyanobacterial and diatom exudates involve low molecular weight compounds and exopolymeric substances (EPS) mainly composed of sugars and proteins (DECHO 1990, HOAGLAND ET AL. 1993). These compounds play an important role in microbial mats, being involved in different adaptive strategies. They are implicated in the process of surface colonization via chemical bonding mechanisms (WETHERBEE ET AL. 1998; DECHO 1994), as well as in nutrient accumulation (WOLFAARDT ET AL. 1998) and desiccation resistance (HOAGLAND ET AL. 1993). Since the transport of solutes inside the mat is largely controlled by diffusional mechanisms (DE BEER AND KÜHL 2001), EPS play an important role in controlling diffusion processes between the mat matrix and the underlying water. The EPS also act as a defensive mechanism against protozoa grazing (PAJDAK-STÓS ET AL. 2001). Moreover, under conditions of nutrient deprivation, microorganisms can expel carbon by means of EPS production when carbon fixation is unbalanced

with other nutrients (OTERO AND VINCENZINI 2004). Most of these stresses are present in polar regions.

Studies concerning different aspects of microbial mats from the continental region of Antarctica are abundant (HOWARD-WILLIAMS AND VINCENT 1989, HOWARD-WILLIAMS ET AL. 1989, BROADY AND KIBBLEWHITE 1991, VINCENT ET AL. 1993a, 1993b, 1993c, FERNÁNDEZ-VALIENTE ET AL. 2001, SABBE ET AL. 2004, HOWARD-WILLIAMS AND HAWES 2007, SUTHERLAND 2009). In contrast, there is little a knowledge about microbial mats from the maritime Antarctic area. The scarce few studies performed in the region focus mainly on taxonomical aspects (VINOCUR AND PIZARRO 1995, 2000), whereas the physiological functioning of these communities remained undefined. These taxonomical studies showed microbial mats from maritime region to be more diverse compared to those from continental areas. Consequently, microbial mats from maritime region are expected to show a greater range of physiological trends than microbial mats from continental areas.

Also important are studies underlining the ecological role of microbial mats in the functioning of freshwater food webs and nutrient cycling. Some research performed in the continental region of Antarctica demonstrates how microbial mats exceed the contribution of planktonic primary production to carbon metabolism (SABBE ET AL. 2004, MOORHEAD ET AL. 2005). Therefore, investigating their functional significance in primary production and nutrient cycling will improve our knowledge of biogeochemical cycles in polar regions. Some of the mats studied in this chapter are located in the catchment of Lake Limnopolar and are expected to be an important allochthonous source of nutrients via runoff. Similar studies in the Arctic suggested a major role of microbial mats in supplying food resources to maintain the pelagic production (RAUTIO AND VINCENT 2006).

The diazotrophic metabolism might also account for nitrogen inputs in regional freshwater ecosystems. For instance, N_2-fixation in cyanobacterial mats in ponds from the McMurdo Ice Shelf area were shown to be the major N input in this ecosystem (FERNÁNDEZ-VALIENTE ET AL. 2001). Likewise, a low supply of combined nitrogen, which based on previous chapters could be a regular trend in Byers ecosystems, might prove advantageous for cyanobacteria with this enzymatic capability. In general, these communities dominated by non-heterocystous cyanobacteria fix nitrogen predominantly during the transition from dark to light and vice versa during minimums of oxygenic photosynthetic production (VILLBRANDTA ET AL. 1990) because a virtually anoxic environment is required for the correct functioning of the nitrogenase enzyme. However, when heterocystous cyanobacteria

are dominant or sub-dominant significant rates of nitrogen fixation can be measured at full sunlight (FERNÁNDEZ-VALIENTE ET AL. 2001).

In this chapter, we report a characterization of the most representative microbial mats observed on the Byers Peninsula to establish their ecological role in the productivity of freshwater ecosystems in the site. Early limnological surveys carried out in Byers (SCAR 2003) showed they are relatively abundant in different habitats of the peninsula, showing a certain degree of variability depending on location. Three different microbial mats representing the variability observed in the region were chosen for a comparative study on structure and functionality, with analysis focused on the phototrophic organisms and their activities. A combination of different techniques was employed, including morphometric characterization of specimens by microscopy and biochemical and physiological methods, including the experimental determination of inorganic carbon and combined nitrogen assimilation and N_2 fixation. In addition, this study assessed photosynthetic activity by means of microelectrode techniques.

7.2. Methodology

7.2.1. Microbial mats sampling

A major part of the sampling as well all physiological experiments with microbial mats was carried out during summer 2001-02. For all analytical determinations, samples were cored randomly using a metal core-taker with a diameter of 13 mm. Experiments and analyses during this summer were made with mats as a whole, that is, maintaining the integrity of vertical structure intact. At summers 2002-03 and 2003-04, further samples of the same types of mats were collected to perform a study of their vertical structure. In this case, they were sectioned in the two distinctive layers that compose the mat and stored separately for analyses.

The analytical study of the mats involved the quantification of the photosynthetic pigments content by HPLC, exopolymeric substances (EPS), isotopic signature and stoichiometry (for detailed procedures see chapter 2). In all cases, samples were placed in sterilized Whirl-pak® bags and kept frozen until analysis. For microscopic examination, cores were conserved in formalin (4% final concentration) in scintillation vials. These samples were examined to determine their taxonomic composition. The taxonomical affiliation of cyanobacteria and diatoms was carried out based on their morphological properties using appropriate keys.

Thus, the classification system of ANAGNOSTIDIS AND KOMAREK (1988) and KOMAREK AND ANAGNOSTIDIS (1989) and the morphotype analysis of BROADY AND KIBBLEWHITE (1991) were used for taxonomic identification of cyanobacteria (Table 7.1). Diatoms were classified following GERMAIN (1981) and KRAMMER AND LARGE-BERTALOT (1986, 1988, 1991). In any case, specimens were identified generally to genus, and to species level only when possible.

Tabla 7.1 *The classification system of cyanobacterial groups based on the morphotype analysis of BROADY AND KIBBLEWHITE (1991).*

Morphotype	Trichome shape in cross section	Presence of calyptra (thickened membrane) on mature apical cell	Number of trichomes in sheath	Trichome shape	Terminal attenuation of trichome	Shape of apical cell	Constrictions at transverse walls	Range in width of thricomes	Range of lenght cell	Ratio of lenght cell to thricome width
A	Cylindrical	(+)	1 or no sheath	Mostly straight often with a slight terminal hook	Slight over final 2-8 cell	Broadly rounded to slightly attenuated to slightly conical	None	2.7-3.2	1.4-4.7	0.5-1.5
B								3.6-5.4	1.6-8.5	0.5-1.75
C								5.4-6.4	1.6-5.7	0.3-1
D								6.9-8.2	2.7-7.3	0.4-0.95
E							Indistinct	8.2-10.9	2-6.6	0.25-0.6
F								12.2-16	3.5-7.4	0.25-0.5
G			Many					2.3-3.6	1.2-5.9	0.25-2
H								3.6-5.4		
I			No sheath		Apical cell only	Attenuated often cuved	None	1-1.5	2-4.9	1.6-3.8
J		(-)	1, rarely 2-3	Straight	None			2.2-3.6	2.5-6(-9)	1.3.5
K								4.1-6.4	1.2-4.5	0.2-0.9
L								5.4-7.8	1.6-6	0.3-1
M			1 or no sheath	Mostly straight often with a slight terminal hook	Slight over final 2-8 cell		Indistinct	8.2-10	1-3.5	0.1-0.38
N	Markedly flattened, narowly ellipsoidal			Straight	None		Distinct	15-23	1.5-3.5	0.08-0.2
O	flattened broadly ellipsoidal			Helically coiled				11-14	1.5-4.5	0.01-0.35

Table 7.2. *Different experimental conditions assayed for the measurements of inorganic carbon uptake in microbial mats.*

Treatment	Setting	Processes measured
Light	exposed to ambient light	Oxygenic photosynthesis + Anoxygenic photosynthesis + Chemolithotrophy
DCMU	exposed to ambient light + DCMU (10µM final concentration)	Anoxygenic photosynthesis + Chemolithotrophy
Dark	deprived of light	Chemolithotrophy
Blank	deprived of light + formalin (4% final concentration)	Passive precipitation

Incubations were maintained for 2 h, and they were subsequently stopped by adding 1 ml of 1N HCl. The bags were then opened to allow non-fixed carbon to escape as $^{13}CO_2$ gas. After neutralizing samples with 1 ml NaOH 1N, the water inside the bags was discarded. Cores were cleaned three times with Milli-Q grade water and then preserved in darkness at -20 ºC. The ^{13}C enrichment in samples was determined in a IRMS Micromass-Isochrom mass spectrometer as described in section 2.2.9. With the results obtained, the rate of carbon uptake (ρC) was obtained using the following equation:

$$\rho C(mgC \cdot h^{-1}) = POC \times Vc \qquad \text{(Equation 7.1)}$$

where POC is the particulate organic carbon content on sample in mg (see section 2.2.8 for analytical procedure) and V_C (h^{-1}) is the specific incorporation rates of carbon obtained with the next equation:

$$V_C(h^{-1}) = \frac{\%^{13}C_m - \%^{13}C_{ab}}{\left[\frac{^{13}C_0}{^{13}C_0 + DIC} \times 100\right] - \%^{13}C_b] \times t} \qquad \text{(Equation 7.2)}$$

where $\%\ ^{13}C_m$ = percentage of ^{13}C in the sample after incubation; $\%\ ^{13}C_{ab}$ = percentage of natural abundance ^{13}C in mat before experiment; $\%\ ^{13}C_0$ = percentage of ^{13}C added to bags; $\%\ ^{13}C_b$ = percentage of ^{13}C present in the controls; DIC = dissolved inorganic carbon in water used for incubations (mg); t = incubation time

(hours). The concentration of dissolved inorganic carbon (DIC) in the incubation water (as needed for calculations) was determined from total alkalinity by pH (see section 2.2.5 for details). All results are expressed as the average of three replicates.

To know the relationship between the photosynthetic activity and the photons flux (*P vs. I*), an irradiance gradient was performed with a log series of accumulated screens. The results obtained were fitted to the hyperbolic tangent function (PLATT ET AL. 1980) showed in the next equation:

$$P_n = P_{\max} \cdot (-\alpha \frac{I}{P_{\max}})^{-Ek} \qquad \text{(Equation 7.3)}$$

where P_n and P_{max} are the net rate of photosynthesis and the maximum rate of photosynthesis respectively, both expressed as μg C (mg Chl-a^{-1}) h^{-1}, *Ek* is the luminous intensity at which the rate of carbon assimilation (μmol photons · m^{-2}s^{-1}) saturates, and α is the initial slope of the function [(μg C (mg Chl-a^{-1}) h^{-1})·(μmol photons m^{-2} s^{-1})$^{-1}$].

7.2.2. Measures of oxygen profiles and gross photosynthesis with microelectrodes

Oxygen microelectrodes were used to perform photosynthetic activity measures without seriously disrupting the mat community. The vertical profiles of O_2 saturation in mats were obtained using a Clark type oxygen electrode (Diamond General. Ann Harbor, Mi, USA; Figure 7.1) with a cathode made out of platinum and the anode elaborated of silver. The method is based in the transformation of O_2 present in sample to hydroxyl ions via chemical reduction with electrons originated from cathode. The voltage signal that generates this reaction was standardized with a two points linear calibration, that is, 0% and 100% oxygen saturation obtained with distilled water purged with N_2 and air saturated with O_2 respectively. To carry out profiles, microelectrode was held handily using a micromanipulator attached to a heavy solid base, which allowed introducing the electrode inside the mat and obtaining measures at intervals of 200 μm.

The gross rates of photosynthesis were measured with the microelectrode following the methodology originally described by REVSBECH AND JØRGENSEN (1983). The method is based on the measurement of change in the concentration of oxygen within the mat after inhibit photosynthesis with darkness following a

prolonged period of exposure to light. In a steady state, O_2 concentration is a balance between the photosynthetic production and the consumption due to respiration and diffusion processes. When photosynthesis is inhibited by dark, during few seconds the rate of the losses processes (i.e., respiration and diffusion) does not vary; the drop in O_2 concentration measured in a layer of the mat is therefore proportional to the photosynthetic production (REVSBECH 1981). Measures were made indoors at constant light of 750 µmol photons m^{-2} s^{-1} provided with a halogen lamp. The mode of operation was to measure at intervals of 200 µm in the vertical profile with the micromanipulator, registering the drop in O_2 concentration (=slope) within the 4 seconds after switching-off light. Photosynthetic rates can then be calculated from the equation 7.4, which is based on a one-dimensional model, assuming horizontal homogeneity.

$$P_t(x) = \int_{-\infty}^{\infty} P_0(y)\cdot(4\pi D_e t) - 0{,}5\exp\left(-\frac{(x-y)^2}{4D_e t}\right)dy \qquad \text{(Equation 7.4)}$$

where $P_t(x)$ is the rate of decline of oxygen at depth x in the instant t after darkness, $P_0(y)$ is the production of oxygen at depth and when t=0, and D_e is the coefficient of effective dissemination of O_2 in the mat that is obtained with the next equation:

$$D_e = \Phi^2 D_o \qquad \text{(Equation 7.5)}$$

where D_0 is the coefficient of molecular diffusion of O_2 in a water pure solution (=2.09 $\cdot 10^{-10}$ cm^2 s^{-1} to 20 ℃) and Φ the porosity of sediment, which was obtained from the relationship between the volume of water contained in mat and the its bulk volume.

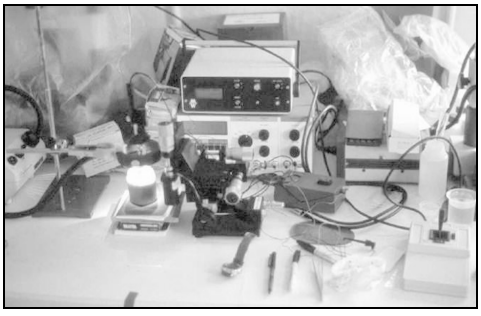

Figure 7.1. *Polarographic microelectrode Clark type and micromanipulator used in the study of microbial mats from Byers Peninsula.*

7.2.3. Setting of $^{15}NO_3$ y $^{15}NH_4$ uptake assays

The uptake rates of combined inorganic nitrogen was measured in mats by the stable isotopic method using $K^{15}NO_3$ and $(^{15}NH_4)_2SO_4$ respectively (FRENETTE ET AL. 1996). The procedure to prepare samples was common to that described in section 7.2.2 for carbon uptake, although only light and dark treatments were performed in this case. To start the experiments, bags were filled with cores and 10 ml of GF/F filtered water. After they were complemented with a volume of a concentrated solution of $K^{15}NO_3$ or $(^{15}NH_4)_2SO_4$ (99% and 98% of ^{15}N atoms respectively) such that the proportion of isotope resulted in around 10% of total dissolved inorganic nitrogen (DIN) present in water. Following, incubations were performed as described in section 7.2.2. After two hours, the incubations were stopped by discarding water from bags and washing the cores three times with Milli-Q grade water to eliminate the exceeding non incorporated ^{15}N. After this, the cores were kept at -20 °C in darkness until analysis. Similarly to carbon uptakes, all experiments were performed by triplicate and conducted around noon. Once in the lab, the ^{15}N atom enrichments of samples were determined by mass spectrometry as described in section 2.2.9. The rate of nitrogen uptake (ρN) was then obtained from the following equation:

$$\rho N(mgN \cdot h^{-1}) = PON \times V_N \qquad \text{(Equation 7.6)}$$

where *PON* is the particulate organic nitrogen content of sample in mg (see section 2.X for analytical procedure) and V_N the specific rate of nitrogen incorporation (t^{-1}) obtained from the next equation:

$$V_N(h^{-1}) = \frac{\%^{15}N_m - \%^{15}N_{ab}}{\left[\frac{^{15}N_0}{^{15}N_0 + DIN} \times 100\right] - \%^{15}N_b] \times t} \qquad \text{(Equation 7.7)}$$

where $\%\ ^{15}N_m$ = percentage of ^{15}N present in the sample after incubation (see section 2.2.10 for analytical procedure); $\%\ ^{15}N_{ab}$ = natural abundance of ^{15}N in mat; $\%\ ^{15}N_0$ = percentage of ^{15}N added to the sample; $\%\ ^{15}N_b$ = percentage of ^{15}N present in control; DIN = dissolved inorganic nitrogen in water from the site used for incubations (mg); t = incubation time (hours). All results are expressed as the average of three replicates.

7.2.4. Analysis of the acetylene reductive activity (ARA)

Dinitrogen fixation in mats was estimated as the ethylene production due to acetylene reduction activity (ARA) in the mat (CAMACHO AND DE WIT 2003). Three cores from each mat were placed in plastic flat bottles with 60 ml of GF/F pre-filtered mat overlying water from the site. Following, bottles were sealed with reversible rubber stoppers and parafilm to avoid gas losses. To start incubations, 10% of the air inside bottles was replaced with acetylene gas freshly generated from calcium carbide (CaC_2) using Vacutainer™ precision glide needles. The air removed was conserved in vacutainer vials to establish ethylene initial amounts. After four hours of incubation a gas sample was collected in the same way to establish the final concentrations. Before obtaining samples from the bottles, they were shaken vigorously to equilibrate the acetylene and ethylene concentrations inside them. Once in the lab, ethylene concentration was determined on duplicates with a gas chromatograph Shimadzu® model GC-8A equipped with an ionization flame detector and a column Porapak® N80/100. Calculations were made by considering that 15,000 counts are equivalent to 10 nmol ethylene. Data were also corrected on the basis of the percentage of acetylene losses.

7.3. Results

7.3.1. Macroscopic characteristics and distribution of microbial mats

The figure 7.2 shows the location of different areas where microbial mats were collected. They were widespread in the region, in some locations forming large areas up to several hundred m^2. They were found also coating streams and shallow lakeshores. The diverse communities observed showed different thicknesses, colours, and degrees of vertical stratification. The microbial mats with high vertical stratification lined puddled soils in which there was limited water availability. They presented an upper dark purple or black layer and a deep green basal layer in which microorganisms alternated with fine-grained sand and mineral deposits (Fig. 7.3a and 7.3b). These mats were habitually observed in the catchment area of lakes from the upland and had a thickness around 3–4 mm. They were found attached to stones and grains of soil and showed a non-uniform and wrinkled surface following the microtopography of the gravel underneath. This community was named **soil mat** and will be referred to as such in this text.

A somewhat similar mat type was also found in the uplands of central plateau. This community was about 4-5 mm thick and coated the ephemeral pond produced by water retention. In contrast to the soil mat, however, this mat was dark orange-brown in colour and had a smooth surface and cohesive texture (Fig. 7.3c and 7.3d). This community was named **pond mat**. By contrast, benthic communities associated with running waters were thicker with lesser vertical zonation than the previous. These less cohesive mats were observed predominantly in the lotic ecosystems or along the margins of lakes, especially shallow lakes. In general, these mats showed a green or yellowish-brown surface and varied in thickness between 2 mm and 3 mm (Fig. 7.3e and 7.3f). This community was named **stream mat**.

Additionally, some lotic environments showed a notable diversity of benthic communities compared to sites described previously. In the main channel of some streams, annual biofilms dominated by green algae prevailed, indicating they are adapted to faster flowing conditions. On the contrary, perennial communities with a macroscopic aspect similar to communities previously described appeared in these sites under a variety of conditions. These stream biofilms coated margins and rivulets, thriving when partially submersed in stagnant waters but also in sites with a lower water availability. Surface appearances were flaked with a leathery consistency. A study of these particular stream biofilms is presented in the next chapter. The present chapter is restricted to the study of the three mats mentioned above.

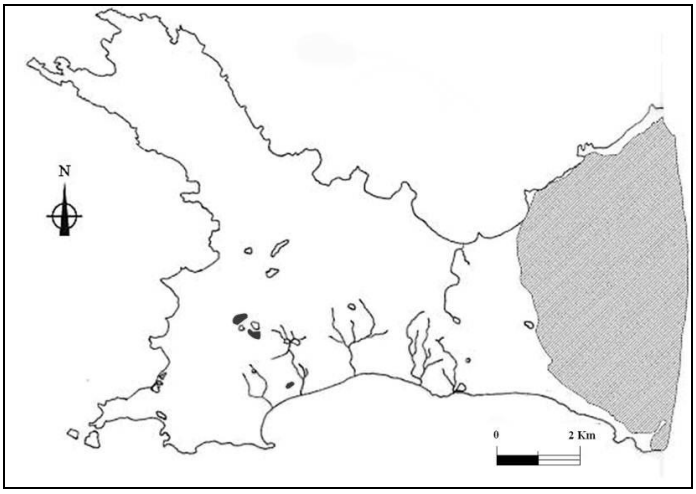

Figure 7.2. *Map showing the locations (black areas) in which microbial mats where more extensively sampled.*

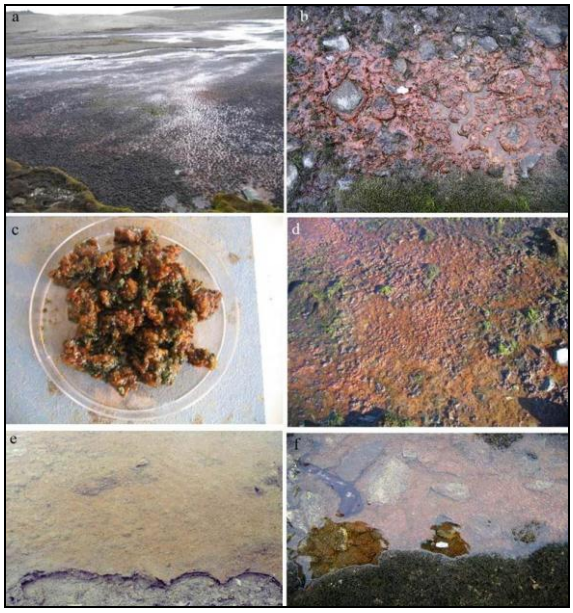

Figura 7.3. *Pictures of three microbial mats studied in Byers Peninsula. a-b) soil mat; c-d) pond mat; e-f) stream mat.*

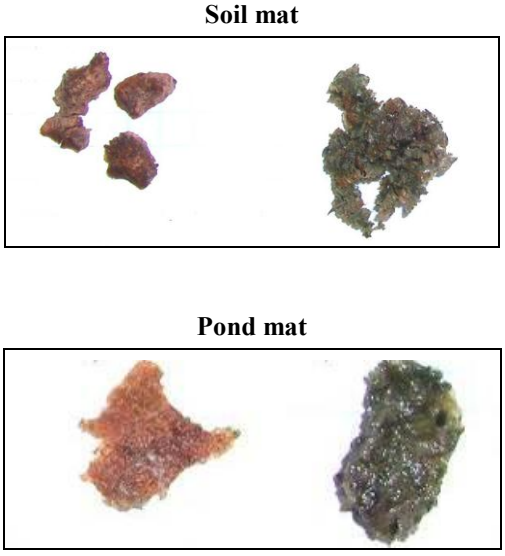

Figure 7.4. *Pictures of surface (left) and basal (right) layers of two microbial mats studied in Byers Peninsula. Differently to these mats, the layering of the stream mat was not easily discernable.*

7.3.2. Taxonomic composition of phototrophic communities

Optical microscopic observations confirmed composition differences among the studied mats (Table 7.3). In all cases, phototrophic communities were comprised mainly of cyanobacteria and diatoms; chlorophytes were also present in some communities, although to a lesser extent. Mats harbored a variety of recognizable cyanobacterial types (Fig. 7.5). Both heterocystous and nonheterocystous cyanobacterial taxa were observed in mats, which the latter dominated regularly. The commonly occurring genera of diatoms were *Navicula*, *Fragilaria*, *Stauroneis*, *Nitzschia*, *Gomphonema* and *Pinnularia*, whose pictures are shown in figure 7.6.

The stream mat contained a high density of diatoms that accounted for up to 70% of phototrophic biomass. Diatoms were particularly abundant in the upper layer of the mat. Filamentous cyanobacteria of different filament widths, members of the Oscillatoriales, were the next dominant and were preferentially located in the bottom layer. Most of the cyanobacterial biomass in this mat consisted of a thin (1–1.5 mm in diameter) cyanobacterium of morphotype I class, similar to that described by BROADY AND KIBBLEWHITE (1991) concerning oscillatorian diversity in the Ross Island and Southern Victoria Land in continental Antarctica. Thicker cyanobacteria from morphotypes J (2–2.5 mm in diameter) and C (5.5–6 mm in diameter) were also present. According to ANAGNOSTIDIS AND KOMAREK (1988), all of these morphotypes could be assigned to different species of the genus *Phormidium* (BROADY AND KIBBLEWHITE 1991). Filaments that were 2–2.5 mm in diameter with a clear partition assigned to the genus *Pseudanabaena* were also present. No heterocystous cyanobacteria were observed in this mat.

In contrast with the stream mat, diatoms were scarce in the soil mat. The matrix of this mat was formed by very thin filamentous cyanobacteria belonging to the genus *Leptolyngbya* (diameter 0.7–1 μm), which accounted for most of the biomass. In addition, a wide diversity of Oscillatoriales was found in this mat, with diameters ranging from 2 to 8 mm and belonging to morphotypes C (diameter, 5.5–6), E (diameter, 8–11 mm), J (diameter, 2–3.5 mm), K (diameter, 4–6 mm) and M (diameter, 8–10 mm) (BROADY AND KIBBLEWHITE 1991). A filamentous cyanobacterium with a dark brown thick sheath (18 mm in diameter) was abundant in the surface layer of the mat. Cyanobacterial cells 5.5 mm in diameter could sometimes be observed emerging from broken sheaths. This cyanobacterium has not been conclusively identified as yet, but it likely belongs to the family Phormidiaceae, genus *Porphyrosiphon*, due to its thick lamellated and colored sheath. A large number of green and brown microcolonies of heterocystous cyanobacteria from genus *Nostoc* were observed in the bottom layer of the mat.

The pond mat consisted of a matrix formed by two types of filamentous cyanobacteria with diameters of 1 and 3 mm and assigned to morphotypes I (1 mm in diameter) and J (3 mm in diameter). An unicellular cyanobacteria (1.5 mm in diameter) was intermixed with the filaments. Diatoms were also present but in low density. Much like the soil mat, filamentous cyanobacterium with a dark brown thick sheath were also present in the surface layer of the mat. Different *Phormidium* species (morphotypes B and K, 4–5 mm in diameter, and morphotype E, 11 mm in diameter) and abundant microcolonies of *Nostoc* appeared in the deepest layer. Pigment composition analyses of the mats revealed differences among them (Table 7.3). The stream mat had a higher chlorophyll-*a* (Chl-*a*) content relative to the surface area, followed by pond mat. For chlorophyllic derivatives, pheophytin-*a* was notably higher in the pond mat, whereas the stream and soil mats had low and similar values. In terms of the relative content of xanthophylls, diatoms (fucoxanthin) dominated in the stream mat, whereas cyanobacteria (myxoxanthophyll) were much dominant in the soil and pond mats. Specific biomarkers for green algae (lutein) were only found in the stream mat, though at low concentrations compared with other carotenoids.

7.3.3. Biomass, stoichiometry and exopolymeric substances (EPS)

The three studied mats differed in water content, biomass and elemental composition, values whose are summarized in Table 7.3. The most notable differences were between the soil mat and the other mats. The soil mat community showed significantly lower values for fresh weight ($p=0.024$); dry weight ($p=0.019$); ash-free dry weight ($p= 0.017$); however it showed significantly higher values for water content ($p=0.036$); and EPS-carbohydrates content ($p=0.002$). The stoichiometric composition of the soil mat showed the highest C and N content per unit biomass ($p=0.002$ and $p=0.001$ respectively) compared to the others. On the contrary, the stream mat showed higher P content than the other two mats, although differences were only significant with respect to the soil mat ($p=0.0004$). As a result, all molar ratios of carbon with respect to N and P, as well as the N/P ratio, were significantly higher in the soil mat than in the others.

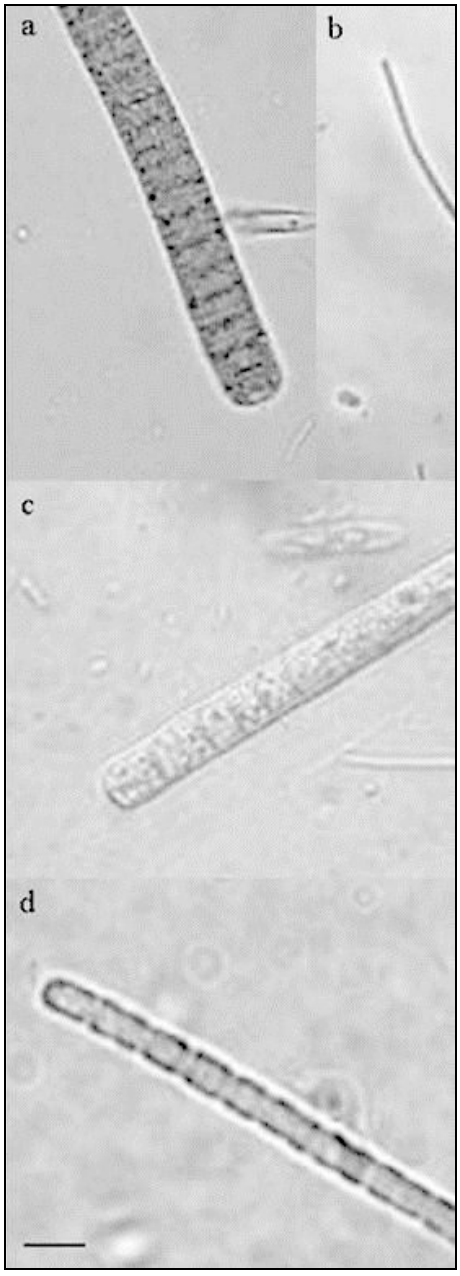

Figure 7.5. *Common cyanobacterial morphotypes found in the microbial mats of Byers Peninsula. a) Oscillatoria sp. b) Leptolyngbya sp. c) Phormidium* cf. *autumnale , and d) Pseudoanabaena sp. Scale bar indicates 10 µm.*

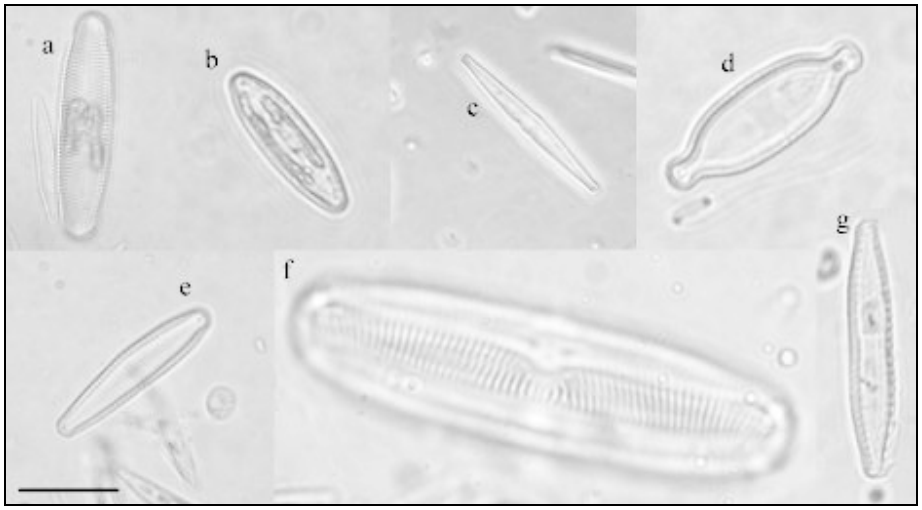

Figura 7.6. *Pictures of more abundant diatoms observed in microbial mats of Byers Peninsula. a) Navicula sp. b) Navicula sp. c) Fragilaria sp. d) Stauroneis sp. e) Gomphonema sp. f) Pinnularia sp. g) Nitzschia sp. Scale bar indicates 50 µm.*

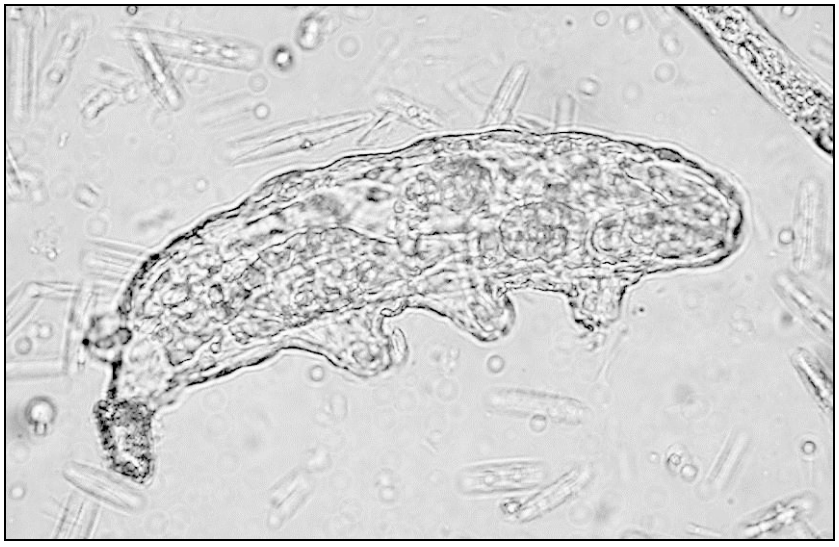

Figura 7.7. *Picture of tardigrade regularly observed in microbial mats.*

7.3.4. *In situ* ^{13}C-bicarbonate uptake measurements

Results for carbon uptake assimilation in the three mats are shown in figure 7.8. In all cases, higher photosynthetic rates occurred under illuminated conditions compared to those obtained in treatments designed to inhibit photosynthetic activity (i.e., +DCMU and dark). However, regardless of the low carbon uptakes measured in these treatments (approximately 8-fold lower with respect to light treatments), the pond mat showed higher rates of carbon fixation compared to the formalin-fixed controls. No detectable activity was observed for DCMU in dark treatments for the other two mats. Under illuminated conditions, specific rates (μg C mg Chl-a^{-1} h^{-1}) were 5-fold higher in the soil mat compared to the other mats ($p=0.009$). However, the photosynthetic rates normalized to the surface (cm^{-2}) showed a different trend. In this case, the soil mat displayed the lower activity while the stream mat showed higher uptakes.

7.3.5. Photosynthetic *versus* irradiance experiments

Parameters yielded from fitting the photosynthetic light–response curves are shown in Table 7.4. The curves obtained for each mat are shown in figure 7.9. Mean irradiances during the assays were 691 μmol photon m^{-2} s^{-1} for the stream mat; 953 μmol photon m^{-2} s^{-1} for the soil mat; and 390 μmol photon m^{-2} s^{-1} in the case of the pond mat. Derived parameters from the curves were P_{max}, which indicates the maximal rate of gross photosynthesis; the slope (α) of the light-limited portion of the curve; and saturation irradiance (E_k), which is the light flux at which α intercepts P_{max}. In agreement with results obtained in the ^{13}C uptake experiment, the maximum photosynthetic activity was observed in the soil mat. Thus, the P_{max} in this mat was 3-fold higher than in the other mats. In the same way, E_k was also higher, although differences were not significant. The values of α were not significantly different between mats.

Table 7.3. *Some characteristics and variables measured in three microbial mats. Data are means ± SD from three replicates. (n.d. = not detected).*

Variable	Unities	Community		
		Stream	Soil	Pond
Location		Margins of lentic ecosystems and rivulets	Soils more exposed to drought	Puddle soils and flat lowlands
Phototrophic assemblage		Diatoms > Cyanobacteria > Chlorophytes	Cyanobacteria >Diatoms	Cyanobacteria >Diatoms
Dominant taxa		*Navicula, Fragilaria, Stauroneis, Nitzschia, Gomphonema, Pinnularia, Phormidium* cf. *Autumnale*	*Leptolyngbya, Nostoc, Oscillatoria, Porphyrosiphon*	*Phormidium* cf. *autumnale, Oscillatoria, Porphyrosiphon, Nostoc*
Fresh weight	mg cm^{-2}	272±99	144±41	338±79
Dry weight	mg cm^{-2}	112±45	33±7	123±32
Ash-free dry weight	mg cm^{-2}	27±11	20±4	44± 11
Water content	%	59±2	77±2	64±5
EPS-carbohydrates	mg g dry wt^{-1}	22.8±2.59	42.52±4.04	27.7±5.06
EPS-proteins	mg g dry wt^{-1}	4.63	16.93	6.26
Carbon	mg g dry wt^{-1}	89.7±21.5	262.7±16.7	129.4±13.3
Nitrogen	mg g dry wt^{-1}	11.2±2.6	16.1±0.5	14.6±2.1
Phosphorus	mg g dry wt^{-1}	2.7±0.8	0.54±0.2	1.8±0.2
C/N	Molar ratio	8.0±0.2	16.3±1.0	8.9±0.5
C/P	Molar ratio	32.7±7.8	486.4±38.9	71.1±7.3
N/P	Molar ratio	4.1±0.9	29.8±2.7	8.0±0.1
$\delta^{13}C$	%	-16.4±0.6	-13.8±0.4	-13.9±1.1
$\delta^{15}N$	%	15.9±2.5	3.9±1.5	4.2±1.0
Chlorophyll-*a*	µg cm^{-2}	61.8±15.4	8.8±2.4	29.0±33.9
Pheophytin-*a*	µg µg Chl-a^{-1}	0.053±0.017	0.064±0.047	15.41±21.25
Fucoxanthin	µg µg Chl-a^{-1}	0.176±0.057	0.094±0.017	0.056±0.007
Myxoxantophyll	µg µg Chl-a^{-1}	0.069±0.019	0.122±0.014	2.091±2.442
Lutein	µg µg Chl-a^{-1}	0.005±0.004	n.d.	n.d.
β-carotene	µg µg Chl-a^{-1}	0.113±0.035	0.2±0.019	0.612±0.655
Scytonemin	µg µg Chl-a^{-1}	n.d.	4.24±0.82	3.18±1.85

7.3.6. Inorganic nitrogen uptake

Measured uptakes for combined nitrogen in the three mats are shown in figure 7.10a (relative to chlorophyll-*a*) and in figure 7.10b (relative to surface) for nitrate, and in figure 7.11a (relative to chlorophyll-*a*), and in figure 7.11b (relative to surface) for ammonium. Under these experimental conditions, the rates of nitrogen assimilation

varied depending on both the substrate and the community. In general, rates were slightly lower in dark versus light treatments. However, these differences were only significant in the case of nitrate. Among communities, the stream mat showed more marked differences between light and dark treatments. With regards to specific assimilation (µg N mg Chl-a^{-1} h^{-1}), NH_4 uptake was significantly higher in all mats than NO_3 uptake in both light and dark treatments ($p<0.002$). The stream mat showed higher incorporation rates of DIN under both conditions, both in the case of nitrate ($p<0.001$) and ammonium ($p<0.001$). However, if specific rates are compared, the soil mat showed the highest rates with both substrates (NH_4 and NO_3) and both treatments (light and dark).

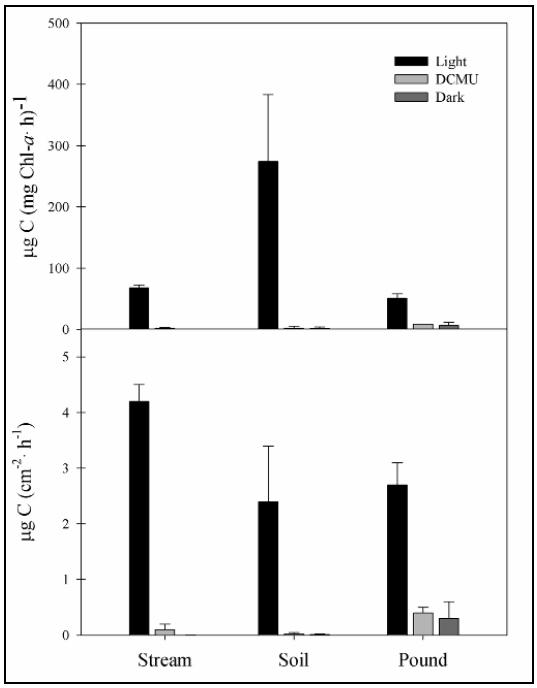

Figure 7.8. *Carbon uptake in three mats at three different conditions: light, light+DCMU and dark. A) rates normalized to chlorophyll-a; B) rates normalized to surface. Bars indicate mean and lines standard deviation.*

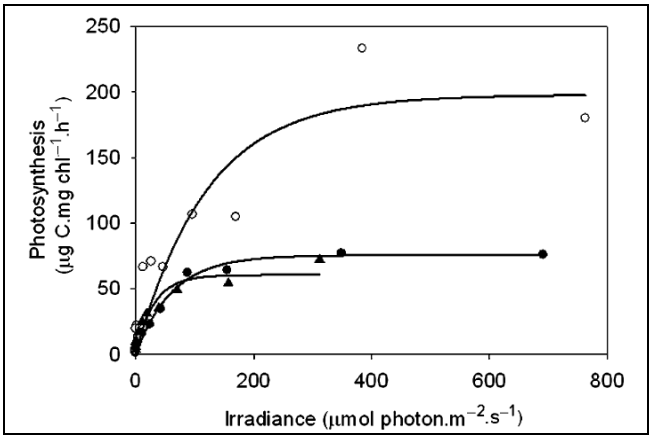

Fig. 7.9. *Photosynthesis vs. irradiance curves in the three studied microbial mats from Byers Peninsula: (●) stream mat; (○) soil mat; (▲) pond mat. Data are means of three replicates. Mean irradiance during the assays were: stream mat, 691 µmol photon m^{-2} s^{-1}; soil mat, 953 µmol photon m^{-2} s^{-1}; pond mat, 390 µmol photon m^{-2} s^{-1}.*

Table 7.4. *Parameters of equations obtained from the fitting of Photosynthesis vs. irradiance (PvsI) curves in three microbial mats.*

Parameter	Unities	Stream	Soil	Pond
P_{max}	µg C fixed (mg chl^{-1}) h^{-1}	75,5±4,7	60,5±12,9	197,7±47,6
α	µg (µg C fixed· mg chl^{-1}h^{-1}) ·(mmol photons · m^{-2}s^{-1})$^{-1}$	1,2±0,2	1,5±0,7	1,3±0,5
E_k	µmol photons · m^{-2}s^{-1}	62,9±12,9	40,3±17,6	152,0±71,7
R^2 (p-value)		0.88 (0.0001)	0.83 (0.0001)	0.88 (0.0001)

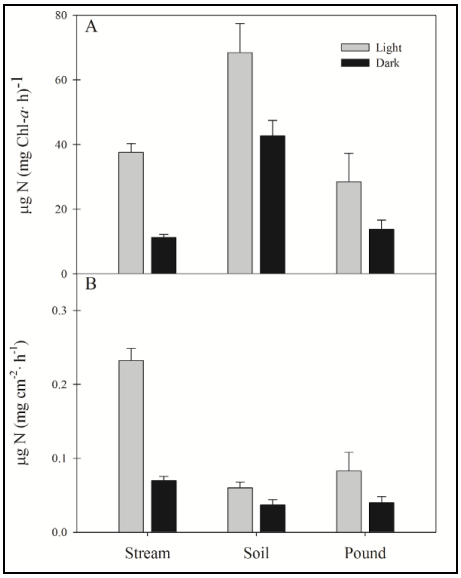

Figure 7.10. *Uptake of NO_3 normalized to Chl-a (A) and surface (B) for the three microbial mats at both light (grey bar) and dark (black bar) conditions. Bars indicate mean and lines standard deviation.*

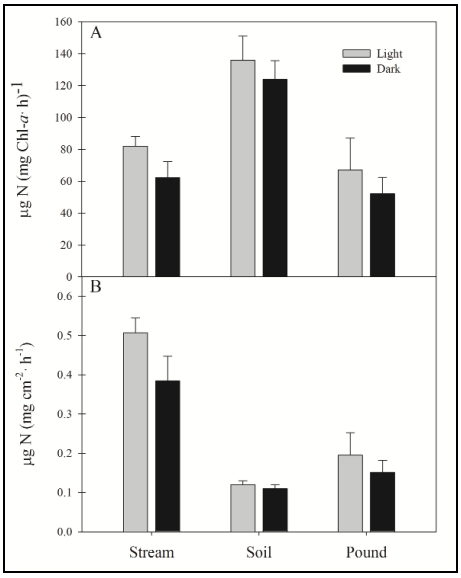

Figure 7.11. *Uptake of NH_4 normalized to Chl-a (A) and surface (B) for the three microbial mats at both light (grey bar) and dark (black bar) conditions. Bars indicate mean and lines standard deviation.*

7.3.7. Acetylene reduction activity (ARA)

In relation to nitrogenase activity, estimated as rates of acetylene reduction (Fig. 7.12), only the pond and soil mats showed a substantial reduction of acetylene under the conditions assayed. Incubations of mats were performed at PAR irradiances of 340, 860 and 275 μmol photons m^{-2} s^{-1} for stream, soil and pond mats, respectively. In the light treatment, the soil mat had higher nitrogenase activity compared to other mats ($p<0.001$). Both the pond and soil mats showed a strong dependence on ARA for photosynthetic activity. Hence, the addition of DCMU and light deprivation resulted in decreased nitrogenase activity. Assuming a conversion factor for acetylene reduced to N_2 fixed of 4 (FERNÁNDEZ-VALIENTE ET AL. 2001), nitrogen fixation in the soil and pond mats under illuminated circumstances entailed 21.4% and a 6.4%, respectively, of the total nitrogen assimilated on these mats.

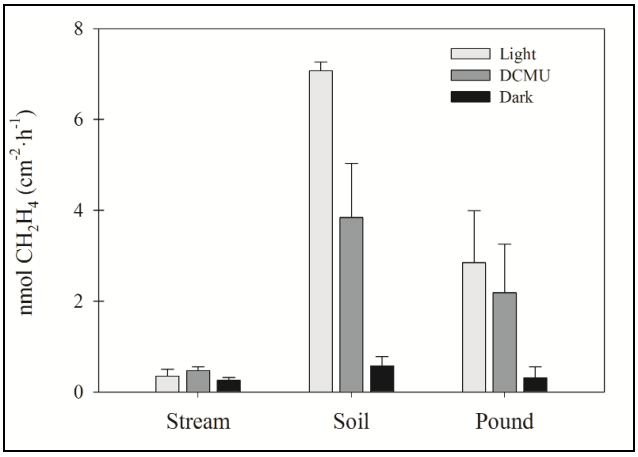

Figure 7.12. *Nitrogenase activity in three mats studied measured as rates of acetylen reduction at different experimental conditions.*

7.3.8. Vertical structure of microbial mats

The chemical analyses showed in sections 7.3.8.1, 7.3.8.2 and 7.3.8.3 were performed only in mats in which layers were easily discernable, which excludes the stream mat (see figure 7.4). With regards to the microelectrodes study showed in section 7.3.8.4, several attempts were made to perform this methodology with the pond mat, but the presence of mineral particles easily broke the glass microelectrode during measurements.

7.3.8.1. Photosynthetic pigments distribution

The Chl-*a* concentration in the mats was higher in the deep layer (Fig. 7.13), although differences were not significant. Values ranged from 0.67-16.15 $\mu g \cdot cm^{-2}$ in the surface layer and from 3.08-14.81 $\mu g \cdot cm^{-2}$ in the deep layer. The photopigment analysis revealed the major presence of taxon-specific carotenoids of cyanobacteria (myxoxantophyll and echinone) and diatoms (fucoxanthin), although green flagellates were detected in some cases (chlorophyll-b and lutein). The carotenoids occurred throughout the mat (Fig. 7.13), but their relative amounts ($\mu g \cdot \mu g$ Chl-a^{-1}) and variation between mats differed greatly with depth. Amounts of fucoxanthin were higher and showed large variation in the lower horizon. Myxoxantophyll demonstrated an opposite pattern to that of fucoxanthin, with higher amounts and variations in the upper layer. On the other hand, the photoprotective pigment scytonemin, when present at all, showed higher quantities in upper layers.

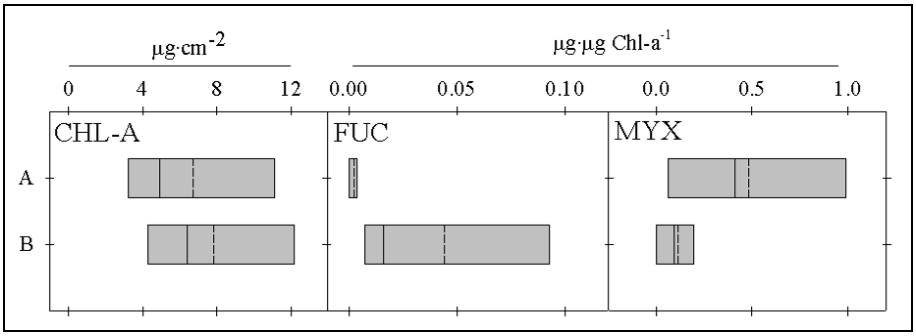

Figure 7.13. *Box plots representing statistical values of the chlorophyll-a (CHL-A) and the major taxon-specific carotenoids (FUC: Fucoxanthin; MYX: Myxoxantophyll) concentrations at layers A = surface and B =bottom of the mats. The boundaries of the box indicate the 25th and 75th percentiles. Solid and dashed lines indicate median and mean respectively.*

7.3.8.2. Stoichiometry

C, N and P content per dry weight in the upper horizon were consistently higher compared to sublayer, though differences were significant only for C and P (Fig. 7.14). Nevertheless, an unbalance was observed in the surface, which revealed a potential P deficiency (Fig. 7.14). Molar ratios of carbon with respect to nitrogen and particularly phosphorus were greater compared to the bottom horizon. In the surface, C/N and N/P molar ratios varied in a similar degree, with C/N ranging from

6.4 to 25.4, and N/P ranging from 3.9 to 94.9. The C/P relationship had a large range, from 30.6 to 2410.9.

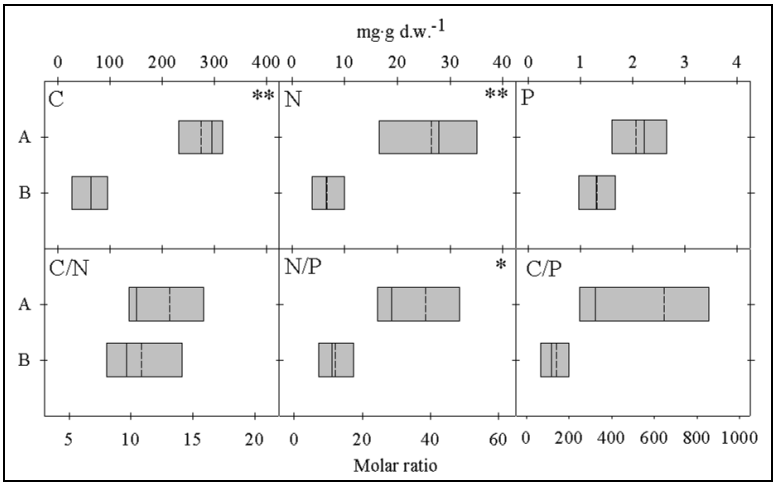

Figure 7.14. *Box plots representing statistical values of the elemental composition (C=carbon, N=nitrogen, and P=phosphorus) and molar ratios at layers A = surface and B =bottom. The boundaries of the box indicate the 25th and 75th percentiles. Solid and dashed lines indicate median and mean respectively. Symbols * and ** indicate significant differences between the mat layers with p>0.05 and p>0.01 respectively.*

7.3.8.3. Exopolymeric substances (EPS) and water content

Moisture in mats varied between layers (Fig. 7.15). Thus water content at the bottom was significantly higher, with values ranging 47-83 %, whereas water content in the surface layer averaged 20 %. All studied mats had exopolymeric substances (EPS) varying from 1-11% of the total dry weight. The protein fraction was considerably lower than the carbohydrate fraction. The distribution with depth for both carbohydrates and proteins is shown in figure 7.15. The EPS-carbohydrates were significantly higher in the surface, where ranged from 53.7 to 117.3 mg·g d.w.-1, while the protein fraction was in average more abundant in the lower layer (range: 2.7-18.6 mg·g d.w.-1). Additionally, EPS content per dry weight was positively correlated (R^2=0.42; p=0.001) with carbon content. In the same way, EPS content was related to taxonomic composition, shwing a significant (R^2=0.69; p<0.001) positive correlation with the relative content of myxoxantophyll, a cyanobacterial specific carotenoid.

7.3.8.4. Vertical profiles of photosynthetic activity

Changes in oxygen concentrations observed during artificial light-dark cycles in the stream and soil mats were significant and reproducible, which allowed estimations of photosynthetic activity on the basis of oxygen evolution. As point out previously the presence of mineral particles in the pond mat it was made impossible to perform reliable measurements.

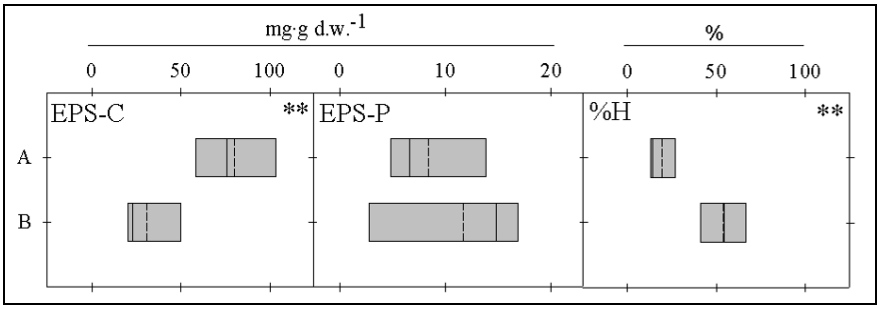

Figure 7.15. *Box plots representing statistical values of the EPS content (EPS-C = carbohydrates, and EPS-P = proteins) and water content (%H) at layers A = surface and B =bottom of the mats. The boundaries of the box indicate the 25th and 75th percentiles. Solid and dashed lines indicate median and mean respectively. Symbols * and ** denote significant differences between the mat layers with p>0.05 and p>0.001 respectively.*

In the two mats for which we obtained data (stream and soil), profiles of photosynthetic oxygen evolution derived from the light-dark shift technique (Fig. 7.16) revealed a pronounced stratification. The photosynthetically active layer was thicker in the soil mat (~4 mm) relative to the stream mat (~3 mm), but the highest maximum activity was found in the stream mat. In both mats, O_2 evolution in the uppermost layer increased with depth until a maximum peak which occurred at different depths in each mat. In the stream mat, two peaks were observed, with upper and higher oxygen production at 800-µm depth and a second lower peak at 2000-µm depth. In the stream mat, these two peaks coincided in depth with maximum relative abundances of diatoms and cyanobacteria, respectively. In the soil mat, the upper and higher peak occurred at a depth of 1600 µm and another area of high O_2 production was detected between 3000 and 4000 µm.

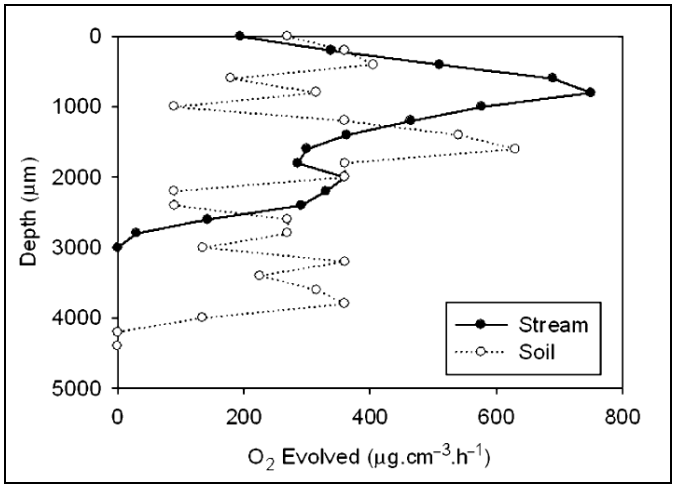

Figure 7.16. *Vertical profiles of oxygen evolution in two microbial mats in Byers Peninsula. Data are means of three replicates.*

7.4. Discussion

The environmental conditions in Byers Peninsula allow the growth and accretion of mat-building microorganisms. Accordingly, they represent a common microbial ecosystem at this site. There are two main limnological areas of distribution in the region. The largest area comprises the central plateau of the Peninsula, where water retention in land depressions has produced freshwater ecosystems with small watersheds. Mats proliferate there, with extreme abundance in the puddle soils of depressed areas and at the bottom of shallow ponds and lakeshores. Mats in this area are submitted to ultra-oligotrophic conditions and sometimes to drought stress. In the coastal areas, by contrast, mats have a more restricted distribution and are confined to streams shores and some flat lowlands. Streams and water bodies in this area show, in some cases, high salt and nutrient concentrations for different reasons (see chapter 3). First, microbial mats coat the downstream areas where the interaction with rock and mineral substrates provokes an increase of dissolved nutrients. They are affected in addition by the proximity of breeding colonies of southern elephant seals (*Mirounga leonina*), penguins and other sea birds. These coastal areas are extensively covered by mosses and the vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunt) Bartl., which impede the expansion of cyanobacterial mats in this region compared to the upland sites.

Although vertical lamination occurs to different extents, depending on the mat and its location, mats typically showed a bi-layer structure which seem to be archetypical of these communities in the region. In all mats, it was possible to identify a top layer comprised of an unstructured stratum, largely composed of empty cyanobacterial sheaths and diatom shells, and a subsurface layer assembled by more competent photosynthetic biomass. Although the uptake of nutrients was not comparatively measured in these two layers, profiles of O_2 evolution performed with stream and soil mats (Fig. 7.16) showed maximum photosynthetic rates at sub-superficial depths, reinforcing the idea that the uppermost layer is essentially non-active. This idea is also supported by of the occurrence of the highest C/N and C/P ratios at this upper layer, which should correspond to diagenetic processes. Beyond the observed nutrient imbalance, the uppermost layer was shown to have a high EPS content and low water content.

The total amount of EDTA-extractable EPS includes both colloidal and capsular fractions, meaning that cyanobacterial sheaths are also obtained during the extraction. This might explain the significant correlation observed between myxoxantophyll and EPS content in mats, demonstrating furthermore a major role of cyanobacteria in the production and uses of these compounds. The EPS determine the cohesive properties of mats, but they are also known to increase the capability to retain water, a consequence of the large amounts of hydrogen bonding in their structure. However, the occurrence of EPS may also be a response to the nutrient scarcity observed in the upper layer of mats. Cell growth is seriously limited when nutrients are scarce, but photosynthetic DIC uptake continues. Cells maintain a relatively balanced elemental composition by driving out secondary metabolites with high carbon content such as EPS, since neither N or P are incorporated in significant quantities into carbohydrates. OTERO AND VINCENZINI (2004) showed experimentally how EPS production in a cyanobacterial strain of *Nostoc* worked as a carbon sink when the C/N relationship in the culture media was high. A cyanobacteria from this genus occurs also in the soil mat, which shows a significant stoichiometric imbalance. In other respects, the EPS might decrease with depth in part due to their microbial utilization. Thus, the constituent macromolecules of EPS can be degraded to mono and dimeric unities by the exoenzymatic activity of bacterial populations, and end products can be then reabsorbed and used again as a inorganic carbon source. Exudates produced by cyanobacteria can comprise both high molecular weight (HMW > 1000 Da) substances, likely aromatic compounds and proteins, and low molecular weight compounds (< 1000 Da) such as simple sugars (KIRKWOOD ET AL. 2006), which all of them are part of the EPS pool.

Both soil and pond mats showed scytonemin in their pigment compositions, but this was not detected in the stream mat. Vertical structure analysis showed that it was concentrated in the uppermost layer. This differential occurrence in mats from the same geographical location indicates that drought an/or nutrient scarcity could exacerbate the effects of UV-B radiation. The synthesis of this UV-screener would be improved under restricted conditions for growth (i.e., low water and/or nutrient availability). In addition, other compounds might screen high energy wavelengths given that β-carotenes and xanthophylls (e.g., lutein, zeaxanthin, fucoxanthin) possess double conjugated bonds that efficiently absorb surface radiation. These carotenoid pigments can also function as antioxidant substances to neutralize reactive species produced by UV photooxidation (PALOZZA AND KRINSKY 1992).

Differences in taxonomic composition, structure and physiological activities of the mats were observed in different locations. This study reflects most, if not all, of the ecophysiological diversity of mats existing in Byers. Among the three mats studied in detail, two were located on the central plateau (i.e., soil and pond mats), and the third was located in the South Beaches (i.e., stream mat). Mats from the upland (soil and pond) shared certain characteristics, such as the relative dominance of cyanobacteria and the presence of sheath pigment scytonemin and higher carotenoids/chlorophyll-*a* ratios. The carbon isotopic signature was similar in these two mats compared to the more depleted delta value of the stream mat. Given that isotopic signatures result from the trade-off of several ecological processes, it is impossible to directly associate them with a particular metabolic group. In any case, certain taxonomical inferences are possible with photosynthetic organisms. Isotopic discrimination is mainly controlled by inorganic carbon (DIC) uptake since isotopic fractionation in the assimilation of reduced carbon is negligible (DES MARAIS ET AL. 1989). This allows differences in $\delta^{13}C$ signatures to be attributed to the mechanism carried out to perform DIC uptake. The microorganisms using the Calvin cycle (cyanobacteria and algae) generate delta values that average around -26 ‰, whereas the chemolithoautotrophic pathway using the reductive citric acid (TCA) cycle imparts a smaller (-10‰) carbon isotope fractionation (ENGEL ET AL. 2004). Additionally, carbohydrates are more enriched with heavy isotopes compared to other biomolecules in photoautotrophs that use Calvin Cycle (VAN DONGEN ET AL. 2002). Consequently, due to cyanobacterial dominance and a higher EPS content in the soil and pond mats, they show depleted values of $\delta^{13}C$.

In general terms, the specific photosynthetic rates (i.e., rates of carbon fixation relative to Chl-*a*) measured in the three mats were low if compared with cyanobacterial communities from temperate environments (ARIOSA ET AL. 2006;

CAMACHO AND DE WIT 2003). These were in the range of measurements for other microbial mats from Antarctica (HOWARD-WILLIAMS AND VINCENT, 1989; HOWARD-WILLIAMS ET AL. 1989; DAVEY 1993; VINCENT ET AL. 1993b) or the Arctic (MUELLER ET AL. 2005). Those measured in stream and pond mats were significantly lower. This is possibly the effect of the accretion of non-functional pigments, which is exacerbated in these mats by the low degradation rates of molecules under cold environmental conditions (VINCENT AND HOWARD-WILLIAMS 1989). Photosynthesis was the primary mode of carbon fixation in the three mats studied, but some distinctive functioning was observed among them. The soil mat showed higher specific photosynthetic rates and values for saturation irradiance (E_k). In relative terms, P_{max} and E_k obtained in PvsI curves in the three mats were lower, and photosynthetic efficiency (α) was higher, compared to cyanobacterial mats from temperate regions (ARIOSA ET AL. 2006), or somewhat higher compared to mats from the Arctic (MUELLER ET AL. 2005). This photosynthetic efficiency, which represents the relative rate of electron transport per unit of light absorbed, is defined by the initial slope of the curves. In other words, this parameter determines the ability to use light at low intensities, meaning mats with higher α values will be more competitive at low light intensities. This parameter is temperature independent since it is ultimately regulated by photochemical processes (MARKAGER ET AL. 1999). This allows for tentative comparisons among different geographical locations. High α values (associated with Low P_{max} and E_k values) are conjectured to be characteristic of shaded environments (BOSTON AND HILL 1991). Because photosynthesis reached its maximal values at sub-surface layers, mats appear to show some degree of adaptation to low light regimes. These findings seem reasonable taking into account the overcast weather conditions of the site. Considering that the maximal oxygen evolution is expected to occur where light conditions are optimal to photosynthesis, it seems reasonable then that O_2 evolution peaked deeper in the soil mat than in the stream mat.

As observed for the carbon assimilation, the soil mat had a higher specific nitrogen uptake rates (i.e., normalized to Chl-a) while the stream mat displayed higher rates normalized to the surface. Among the different substrates, ammonium was the most important nitrogen source for mats. In addition, in all substrates and mats studied, uptake was a photosynthetic-dependent process. This trend has been observed in cyanobacteria (HO AND KROGMANN 1982), which dominate these microbial mats. The assimilation of nitrate involves both nitrate and nitrite reductase enzymes, which consume 8 electrons during the process. In cyanobacteria these enzymes are closely associated with the photosynthetic system, where they obtain reductants needed directly from ferrodoxin linked to PS I (FLORES ET AL. 2005).

This close dependence on photosynthesis could explain the higher assimilation rates displayed under light compared to those observed at dark.

Similar findings were observed for ammonium uptake with regards to light dependence, although in this case it may be regulated in a different way. In contrast to nitrate, ammonium can pass through the plasmatic membrane passively, without an additional requirement of energy, providing it be deprotonated. The balance between protonated (NH_4^+) and deprotonated (NH_3) forms depends closely of pH, with the equilibrium shifted heavily to NH_3 under alkaline conditions. The inhibition of photosynthesis by light deprivation involves a drop in pH values and an increase in the proportion of NH_4. However, other explanations are possible. Because nitrifying bacteria (which promote the oxidation of NH_4 to NO_2 and NO_3) are obligate aerobes, photosynthetic activity (which generates O_2) may promote also nitrification activity in mats.

The contribution of atmospheric-derived nitrogen to the assimilated pool was also notably elevated in the soil mat, which contrast with the extremely low rates observed in the stream mat. This is consistent with the $\delta^{15}N$ values observed in the stream mat since communities dominated by diazotrophic activity are supposed to have an organic N pool depleted in the $\delta^{15}N$ signal (HOLL ET AL. 2007). Streams and rivulets from Byers show relatively high concentrations of combined nitrogen compared to other sites such as lakes and ponds. In contrast, the nutrient deficiency suggested by the C/N ratio in the soil mat might involves a competitive advantage for heterocystous cyanobacteria and therefore explain the higher nitrogenase activity in this mat.

A remarkable outcome from the acetylene reducing activity (ARA) assays is that both the addition of DCMU and the dark treatment substantially reduced fixation rates in both the soil and pond mats. Hypothetically, nitrogenase activity would increase when DCMU is added because oxygen evolution is stopped, although a reduction in N_2 fixation after long exposure to DCMU or dark conditions has been also observed (DUBOIS AND KAPUSTKA 1983; PAERL ET AL. 1994; EVANS ET AL. 2000). One explanation for the deactivation of this enzyme is that the exporting of fixed carbon from vegetative cells to heterocysts might be prevented by DCMU-induced inhibition of photosynthesis, resulting in a total consumption of photoreductants (PAERL ET AL. 1994). This dependence of ARA on photosynthesis has been verified in microbial mats from ponds in the McMurdo Ice Shelf region (FERNÁNDEZ-VALIENTE ET AL. 2001). Given the results obtained in the P*vs*I curves, N_2 fixation experiments were possibly carried out at suboptimal irradiances. For this reason, results obtained in the ARA experiments must be interpreted with caution.

In summary, the combined application of different methodological approaches has revealed the ecological importance and the taxonomic and physiological diversity of microbial mats in this zone from the maritime Antarctica. Our observations show that different environmental conditions result in distinct taxonomic compositions and functions of microbial communities. Variation in species composition, shape, pigmentation and physiological characteristics in microbial mats appears regulated by environmental factors such as nutrient or water availability. Our study has implications in the context of lake ecosystem functioning in the region. Some of the microbial mats studied, such as the soil mat, are located in the catchment area of oligotrophic lakes of the central plateau and can be considered an important allochthonous source of nutrients for the lakes via runoff, thereby contributing to lacustrine foodwebs. These mats are also likely to be important in the successional development of catchment areas because they may contribute to the establishment of mosses, which appear as small colonies intermixed with microbial mats in some areas of the watersheds in the central plateau.

8. Community structure and photosynthetic activity of benthic biofilms

8.1. Introduction

Stream phytobenthic populations represent self-sustaining communities and their primary production and nutrient cycling is crucial in streams. Unlike subglacial channels typical for the continental region of Antarctica, the drainage systems in the maritime Antarctic region are exposed to direct sunlight, permitting photosynthesis. Several studies explored the extensive growth of benthic algal communities during the summer seasons in this area of the continent (HAWES 1989, DAVEY 1993, VINCENT ET AL. 1993a, 1993b, ELSTER AND KOMAREK 2003). However, due to their rarity in Antarctic watercourses, few studies explore the benthic algal growth in cascades or waterfalls. As a consequence of the continuous climate warming in the maritime Antarctic region (QUAYLE 2002, VAUGHAN ET AL. 2003) the occurrence of these waterfalls is likely to increase.

The study performed in the previous chapter reveals the occurrence of perennial and well-developed photosynthetic microbial mats (see chapter 7). Further observations have demonstrated the occurrence also of different biofilm types sparsely distributed in stream channels. A common trend for the thicker microbial mats is the phototrophic microorganisms embedded within a matrix of extracellular polymeric substances (EPS) synthesized by themselves. EPS has long been recognized as an integral part of the structural organization of lithobiontic communities in the McMurdo Dry Valleys (DE LOS RÍOS ET AL. 2004b), where its protective role against the harsh living conditions has been suggested. EPS may act as a multifunctional element, being involved in different adaptative functionalities, as the process of surface colonization via chemical bonding mechanisms (WETHERBEE ET AL. 1998; DECHO 1994), in nutrient accumulation (WOLFAARDT ET AL. 1998), and in offering resistance to desiccation (HOAGLAND ET AL. 1993). EPS production could also be a C release method under unbalanced nutrient concentrations, eliminating part of the photosynthates in nutrient scarcity (OTERO AND VINCENZINI 2004).

The development of these biofilms in fast flowing systems seems adequate to ensure nutrient acquisition. Otherwise, these biofilms appear more dynamic than perennial microbial mats, which supposedly respond rapidly to changing environmental conditions. During snow melting and snow free periods, lotic ecosystems from Antarctic ice-free areas are characterized by hydrodynamic fluctuations marked by highly variable water regimes (INBAR 1995). The discharge occurring during melting periods is thus responsive to climatic variations. Under these circumstances, the fast flow events disturb streams by removing sediments and

by abrading benthic biofilms. In contrast, after the completion of the ice melting, the flow regime decreases considerably and the habitat reconfigures with important changes in water and nutrient availability. In response to this predictable and seasonal cycle, organisms can develop resistance mechanisms or show the capacity to recover from perturbation. Under this scenario of conditions fluctuation, different strategies are required for biofilms to persist during both fast-flowing and ulterior dry periods as well as nutrient starvation. Still, the magnitude and duration of the stress might exceed the tolerance of organisms.

Bottom-up processes are thought to dominate the river ecology. In temperate environments, for instance, the development of different benthic communities in streams is regulated by disturbances such as the hydrological drought (FREEMAN ET AL. 1994, CARAMUJO ET AL. 2008) or nutrient availability (GUASCH ET AL. 1995). However, the factors controlling biofilm growth and distribution remain unknown in Antarctic streams. In this sense, some of the studies mentioned above involve periods covering annual extremes over the course of a year. By contrast, Antarctic streams undergo major changes in shorter periods, which supposedly involve different successional and/or distributional patterns of the biota. The question is whether initial colonization processes occur after the ice melts or whether biofilms are mainly perennial forms with some capacity to recover from perturbations. Diverse biofilms with different adaptative strategies might therefore enhance the resilience of the entire ecosystem.

This chapter provides ecological data on the compositions and activity of photosynthetic biofilms inhabiting a stream of Byers Peninsula, complementing the studies on microbial mats of the previous chapter. Here, we present data on the distribution and photosynthetic activity of five different benthic communities along a cross-sectional transect. The study involved the characterization of the photosynthetic community assemblages, which comprises the quantification of their photosynthetic activities, the exopolymeric substances (EPS), and the isotopic signature and stoichiometric compositions of biofilms. We further investigated from an ecological point of view whether the occurrence of biofilms in this restricted location is mediated by different environmental deterrents, namely the hydrological dynamics associated with thaw periods.

8.2. Methodology

8.2.1. Study site

The study was conducted during February 2002 in a stream sited in the southern beach of Byers Peninsula. In this region, the flow regime of streams is strongly dependent of summer thawing and therefore important fluctuations occur during the hydrological cycle. The stream in which biofilms were growing is the discharge of a lake located on the central plateau of the Peninsula at 65 m a.s.l. (Fig. 1; Turbio Lake). Part of the stream runs through a pronounced slope. During the austral summer, when the water discharge increases due to ice and snow melting, this stream is characterized by a fast water flow forming a cascade, to which follows a rapid of several meters.

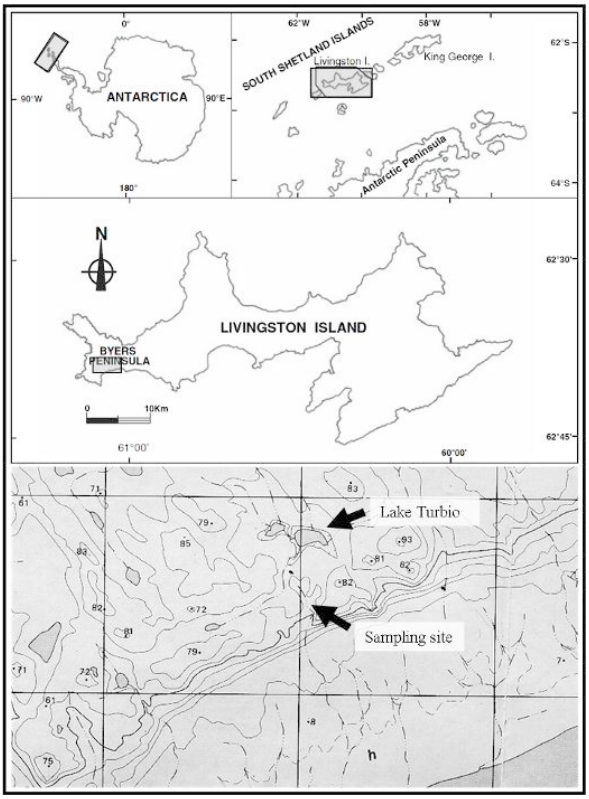

Figure 8.1. *Geographical location (62°34'35"- 62°40'35"S/60°54'14"- 61°13'07"W) of the biofilms studied in Byers Peninsula (Livingston Island).*

8.2.2. Sampling

The occurrence and relative dominance of biofilms in the stream was surveyed along a transect of a few meters (surveyed at a resolution of 5 cm). This transect was chosen as representative of the stream's flow regime variations. Sampling was conducted from the centre of the channel to the shore edge and to sites splashed by the waterfall. Once identified, samples of different biofilm types observed in the stream were randomly collected in triplicate from their representative locations with a metal core taker (18 mm diameter). For the microscopical study, cores were placed in scintillation vials with formalin (4% final concentration) and stored at +4ºC in the dark. Other cores were stored dry at -20 ºC in sterile Whirl-pak bags to perform the rest of the analyses. Conductivity and pH were measured using an YSI® 556 MPS Water Logger System. The samples for inorganic nutrients analysis were collected filtering the water through precombusted glass-fibre filters (GF/F grade) and distributed in acid cleaned polyethylene bottles.

8.2.3. Phototrophic community structure and biomass

The taxonomic affiliation and relative abundances of the different phototrophs observed in biofilms were determined based on morphology. Cyanobacteria were identified following the classification system of ANAGNOSTIDIS AND KOMAREK (1988) and Komarek and Anagnostidis (1989). For microscopical analysis of diatoms small quantities of the samples were cleaned by a modified method of VAN DER WERFF (1955): 37% H_2O_2 was added to samples that were heated to 80 °C for 1 hour. Oxidation of organic material was completed by addition of $KMnO_4$. Following oxidation, the samples were rinsed 3 times with deionised H_2O alternated with centrifugation (10 minutes at 3700 x g). The cleaned material was diluted with distilled water, dried on microscope cover slips, and mounted in Naphrax mounting medium. Samples and slides are stored at the National Botanic Garden of Belgium (Meise, Belgium). In each sample, 400 valves were enumerated on random transects at 1000x magnification under oil immersion using an Olympus BX51 microscope equipped with Differential Interference Contrast (Nomarski) optics. Identifications of Antarctic species are based on KOPALOVÁ ET AL. (2009), SABBE ET AL. (2003), VAN DE VIJVER (2008), VAN DE VIJVER AND MATALONI (2008), VAN DE VIJVER ET AL. (2002, 2010a, b 2011) and ZIDAROVA ET AL. (2009, 2010).

To glean the distribution of photosynthetic pigments in biofilms, a chromatographic (HPLC) analysis of chlorophylls and carotenoids was performed as described in the section 2.3.3 of the thesis. Peak identities were determined by comparing retention times and spectra with those of pure standards purchased from DHI (Denmark). The amount of chlorophyll-a (Chl-a) in biofilms was used as an algal biomass indicator. Myxoxantophyll, fucoxantin and lutein were used as taxa-specific biomarkers for the determination of relative abundances of cyanobacteria, diatoms and chlorophytes, respectively. The quotient between absorbance at wavelengths 480 and 665 nm of extracts was used to estimate the relation between total carotenoids and Chl-a as a potential indicator of nutrient limitation (SCHLTITER ET AL. 1997). Dry weight of biofilms was determined on samples kept frozen after melting and drying at 105 ºC for 6 h.

To obtain the stoichiometric composition (carbon, nitrogen, and phosphorus) of biofilms, carbon, and nitrogen contents were measured by combustion of samples in a CE Instruments EA 1110 CHNS elemental analyzer. The samples were first dried at 60 ºC until obtaining a stable weight. They were then ground to powder in a mortar, weighed over aluminium foil sleeves, and burnt in the elemental analyzer. Gases released from burning were then measured by infrared analysis. The standard was prepared using sulphanilamide. Total phosphorus was obtained with the same method described for SRP after an acid digestion of the dry powder.

8.2.4. Analysis of extracellular polymeric substances (EPS)

The samples to measure the amount of extracellular polymeric substances (EPS) were obtained as described in section 7.2.1 for microbial mats. The analysis was made following the procedure described in section 2.3.2. For the microscopic observation of exo-carbohydrates distribution in biofilms, a portion of the 4%-formalin-fixed samples were dehydrated over glass slides and stained with Calcofluor White ($C_{40}H_{44}N_{12}O_{10}S_2$; λ Excitation max = 347 nm and λ Emission max = 436 nm) for 30 min. Preparations were observed through a Zeiss-III epifluorescence microscopy using the appropriate filter setting for calcofluor fluorescence emission. Several pictures were taken with an Olympus® C-4040 ZOOM camera, and the RGB original images were converted into grayscale.

8.2.5. *In situ* ^{13}C-bicarbonate uptake measurements

The uptake of inorganic carbon in biofilms was measured by the stable isotopic method ($NaH^{13}CO_3$) described as follows. Experimental conditions were designed to discriminate photosynthetic from dark carbon assimilation. Thus, four cores of each biofilm (3 light + 1 dark) were placed in sterile Whirl-pak® bags with 10 ml of filtered (GF/F) water from the site. The experiment was initiated by the addition of a volume of a concentrated solution of $H^{13}CO_3$ (99% ^{13}C atoms) such that the proportion of isotope resulted in around 10% of total dissolved organic carbon in water. Additional cores of each biofilm were incubated in parallel at the same conditions with formalin (4% final concentration) to estimate passive carbon accumulation. All samples were incubated at ambient light intensity (measured with a Li-Cor® LI-193SA) and *in situ* temperature. Incubations were maintained for 2 h, and they were subsequently stopped by adding 3 ml of 1N HCl. The bags were then opened to allow non-fixed carbon to escape as $^{13}CO_2$ gas. After neutralizing samples with NaOH 1N, the water inside the bags was discarded. Cores were cleaned three times with Milli-Q grade water and then preserved in darkness at -20 ºC. Once in the lab, the isotopic enrichment during uptake experiments of dry cores was measured in an IRMS Micromass-Isochrom mass spectrometer. For a detailed description of uptake calculations, see section 7.2.2 of the thesis. The concentration of dissolved inorganic carbon (DIC) in the incubation water (as needed for calculations) was determined from total alkalinity by pH. To establish comparisons among biofilms, uptake rates were normalized both to the surface and to Chl-*a* content.

8.2.6. Data analysis

Analysis of variance was used to detect significant ($p<0.05$) differences between variables measured in biofilms. To identify major patterns of variability among biofilms, a Principal Component Analysis (PCA) was conducted with a total of 15 variables measured in biofilms. Variables were log-transformed ($\log_{10}$ 1+x) to linearize the relationships and avoid the influence of magnitude. A categorical data matrix was created considering the relative abundance of different taxonomical groups in biofilms (i.e., absent=0, present=1, frequent=2, abundant=3). A hierarchic-agglomerative cluster analysis based on the UPGMA (unweighed pair group average linkage method) with the Euclidean Distance as a dissimilarity measure was performed (MVSP 3.13p, Kovach Computing Services 2002).

8.3. Results

8.3.1. Stream chemistry

The main physical and chemical parameters (average ± standard deviation) observed in stream water at the sampling date are summarized in Table 1. In some cases it is shown a mean value that represents the range of variation occurring along the area of biofilms distribution (N=3). Both conductivity and inorganic nutrient concentrations were low and typical of a low mineralization and oligotrophic polar stream, mainly when transit through catchments is relatively short as takes place in this case. The molar ratio of dissolved combined nitrogen (NO_3 + NO_2 + NH_4) and SRP were close balanced (around 15).

Table 8.1. *Main physical and chemical parameters of the stream water. For those parameters displaying a mean value (± standard deviation) it represents the average of three equidistant measures made along the area of biofilms distribution. In the other cases the value represent a single measure made in the central part of the transect.*

Parameter	Unit	Stream water
Temperature	ºC	2.54
O_2	%	98.4
O_2	mg L^{-1}	13.4
Conductivity	µS cm^{-1}	68.00 (± 9.67)
pH		7.22 (± 0.11)
NO_3+NO_2	µM	0.802 (± 0.213)
NH_4	µM	0.856 (± 0.276)
SRP	µM	0.116 (± 0.012)
DIN/SRP	molar ratio	14.3
Silicates	µM	46.91 (± 12.31)
DOC	mg L^{-1}	0.6
DIC	mg L^{-1}	5.48
Cl^-	meq L^{-1}	0.26
SO_4^{2-}	meq L^{-1}	0.28
HCO_3^-	meq L^{-1}	0.65
K^+	meq L^{-1}	<0.05
Na^+	meq L^{-1}	0.57
Mg^{2+}	meq L^{-1}	0.22
Ca^{2+}	meq L^{-1}	0.49

8.3.2. Distribution and physical features of biofilms

Different microphytobenthic communities coated the waterfall faces and the bed of the stream. Visual inspections made *in situ* showed the occurrence of five distinct biofilms with a monolayer structure. Based on macroscopic characteristics (surface colour, shade, roughness), different names were assigned to each: *Deep Black*: DB; *Black-Striped*: BS; *Green*: GR; *White*: WH; and *Orange*: OR. The biofilms showed a patchy distribution in a transect from the centre of the channel to the near-shore stream edge. Moisture varied partly relative to their location. The highest water content was observed in the *Green* biofilm (73%), followed by *Black-Striped* (63%), *Orange* (57%), *Deep Black* (47%) and *White* (40%).

Both *Green* and *Deep Black* were the two biofilms showing the highest extension. The first was on average 2- to 3-mm thick (up to 4 mm) growing preferentially in running water with a dominant distribution in the centre of the stream basin. The *Deep Black* biofilm was thinner (1 mm) showing a more cohesive aspect compared to the former. This biofilm coated extensively the stream shores, where the water flow was lower. The rest of the biofilms grew wherever water flow decreased significantly. The *Black-Striped* biofilm prevailed in sites where the bottom was continuously covered by a fine water layer. This biofilm was similar to *Deep Black* but with darker pigmentation and a thickness of around 0.5 mm. The *Orange* biofilm (2-3 mm thick) was restricted to splashing zones, receiving only intermittent surface moistening. Finally, the *White* biofilm (usually less than 1 mm thick) was associated with sites where the water level was occasionally below the biofilm surface and the flow was nearly negligible. Unlike the other biofilms, this biofilm showed little cohesion and was dismantled easily. Microscopic examination of this biofilm revealed the occurrence of large numbers of nematodes, tardigrades, and several ciliate morphotypes, but the faunal components of these biofilms are out of the scope of this work.

8.3.3. Community structure and photosynthetic activity

Algal biomass, expressed as chlorophyll-*a* (Chl-*a*) content per surface area, was significantly higher in the *Green* biofilm compared to the others (Table 8.2). Algal biomass was similar between *Deep Black*, *Black-Striped* and *Orange* biofilms, whereas *White* showed the lowest photosynthetic biomass. Besides chlorophyll, a diverse set of pigments was also detected with the HPLC analysis, including both pheophorbides and taxa-specific carotenoids. Pheophytin-a, a common degradation

product of Chl-a, occurred in all biofilms, though its ratio to Chl-*a* was approximately 10-fold higher in the *White* biofilm compared to the others.

Table 8.2. *Pigment composition expressed as ratios to chlorophyll-a (wt/wt) in the different biofilms obtained by means of HPLC analysis with purified pigments as standards (DHI, Denmark). Chlorophyll-a is expressed as µg (cm)-2 and phaeophytin-a, fucoxanthin, myxoxantophyll, and lutein as µg (µg chlorophyll-a)-1. (n.d.= not detected). Biofilms are GR (Green), DB (Deep Black), BS (Black Striped), OR (Orange) and WH (White).*

Biofilm	Chl-a	Phaeophytin-a	Fucoxanthin	Myxoxantophyll	Lutein	Car/Chl-a
GR	48.14	0.069	0.364	n.d.	0.174	0.70
DB	25.76	0.023	0.017	0.229	n.d.	1.07
BS	25.82	0.314	0.109	0.061	0.108	1.19
OR	24.45	0.045	n.d.	1.230	n.d.	1.74
WH	9.07	1.124	0.188	0.018	0.146	0.61

Myxoxantophyll, the specific carotenoid of cyanobacteria, occurred in all communities except in the *Green* biofilm, although *Deep Black, Black-Striped*, and *Orange* displayed higher relative contents of myxoxantophyll. Lutein, particular of chlorophytes, was detected in the *Green*, *White* and *Black-Striped* biofilms although was relatively higher in the *Green* compared to the *White* biofilm. In *Black-Striped* biofilms, lutein levels were low, and a possible co-elution with zeaxanthin might have resulted in an overestimation. Fucoxanthin, that occurs only in Bacillariophyceae, was observed in all extracts except in the *Orange* biofilm. This pigment was mostly found in the *White* and particularly in the *Green* biofilm communities; *Deep Black* showed the lowest levels of fucoxanthin.. The total carotenoids/Chl-*a* ratios, estimated as the ratio between 480 and 665 nm in the acetone extracts, ranged from 0.61 to 1.74 (Table 8.2). Biofilms dominated by chlorophytes showed the lowest ratios, whereas the highest ratios were marginally observed in the *Orange* biofilm. On the other hand, ratios in *Deep Black* and *Black-Striped* biofilms were close to 1. UV-protective pigments like scytonemin were not found in any of the investigated biofilms.

A list containing the main phototrophic micro-organisms inhabiting biofilms with their relative contribution, is shown in Table 8.3. A cluster analysis based on these relative abundances (Fig. 8.2) allowed distinguishing the cyanobacterial dominated biofilms (*Deep Black*, *Black-Striped* and *Orange*) from the other two (*Green* and *White*). In the former, the highest similarity was found between *Orange* and *Black Stripped*. To some extent, *Deep Black* represented thus a transition between both groups. The assemblages consisted principally of a variety

of diatoms, green algae and cyanobacteria, with a total of 42 recognized taxa. Except in the *Green* and *White* biofilms, cyanobacteria were common in all communities on different levels. Four different filamentous morphotypes were found and tentatively assigned as *Oscillatoria* ssp. (*Oscillatoria* sp1 5-6 µm width; *Oscillatoria* sp2 9-10 µm width), *Phormidium* cf. *autumnale* (C.Agardh) Trevisan ex Gomont, and *Leptolyngbya* sp. In the *Black-Striped* biofilm, the phototrophic biomass consisted almost entirely of *Oscillatoria* sp1, accounting for 70 to 80% of the total cyanobacterial biomass. *Oscillatoria* sp1 was also abundant in *Deep Black* but co-dominant with other morphotypes. *Oscillatoria* sp2 was less widespread, observed only in the *Black-Striped* and *Orange* biofilms. In the *Orange* biofilm, *Phormidium* cf. *autumnale* and *Leptolyngbya* sp. were present, but the latter was more abundant. *Phormidium* cf. *autumnale* prevailed more in *Deep Black* biofilm than in any other biofilms. In *White* biofilm, cyanobaterial morphotypes were scarce and only *Oscillatoria* sp1 was observed. *Ulothrix* sp. was the most dominant chlorophycean in *Green* and *White* biofilms. Of all the cyanobacterial biofilms, this green alga only occurred in the *Deep Black*. The relative abundances of small flagellates, assigned to the Chrysophycean genera *Ochromonas*, also varied among biofilms, being more abundant in the *Green* and, to a lesser extent, in the *White* biofilm. They sometimes constituted less than l% of biomass in cyanobacterial-dominated biofilms, being more abundant in the *Deep Black*.

A total of 37 diatom taxa were retrieved by microscopical inspection, revealing their variable role in biofilms. They were numerically the most abundant component in *White* biofilm and, to a lesser extent, were present in *Green* and *Black Striped*. Their contribution in the *Orange* biofilm was minor. The cosmopolitan *Fragilaria capucina* s.l. Desm. was the principal species in diatom-dominated assemblages. Other diatoms showed a less cosmopolitan distribution. For example, species such as *Gomphonema signyensis* V.J.Jones & Kociolek, *Nitzschia frustulum* (Kütz.) Grunow and *Psammothidium metakryophilum* (Schmidt & Lange-Bert.) Sabbe were only observed in *Green* and *White* biofilms. Other species such as *Staurosirella pinnata* (Ehrenb.) D.M.Williams & Round, *Nitzschia acidoclinata* Lange-Bert., *Navicula cremeri* Van de Vijver were, by contrast, only observed in *Deep Black* biofilm. In the *White* biofilm, we observed some aerophilic diatoms such as *Hantzschia hyperaustralis* Van de Vijver & Zidarova, *Muelleria australoatlantica* Van de Vijver & Spaulding, *Luticola cohnii* (Hilse) D.G.Mann and *Diadesmis arcuata* (Heiden) Lange-Bert., although their relative abundances were lower compared to other taxa.

The areal rates of inorganic carbon uptake differed significantly among the biofilms under the conditions assayed (Fig. 8.3). The highest rates were observed in *Deep Black*, although they were not significantly different to those displayed by *Green* and *Black-Striped* biofilms. In contrast, significantly lower rates were measured in *Orange* and *White* compared to the other biofilms. No significant differences were observed for the carbon assimilation in the dark treatments versus the formalin-fixed controls, indicating thus that all carbon assimilation measured in the experiment was performed by photosynthetic organisms.

8.3.4. Biomass and stoichiometric composition

The bulk biomass elemental composition (carbon, nitrogen and phosphorus) of the biofilms and their stoichiometric relationships are shown in Table 8.4. Values ranged across approximately one order of magnitude, with the minimum and maximum mean values as follows: 255.21 and 38.84 mg g dw^{-1} for carbon, 36.94 and 3.87 mg g dw^{-1} for nitrogen and 5.20 and 0.99 mg g dw^{-1} for phosphorus, respectively. *Green* displayed the highest and *White* the lowest concentrations of the three nutrients. In biofilms dominated by cyanobacteria, *Deep Black* and *Black-Striped* showed similar values for the three nutrients, but values were lower in *Orange*. The three elements co-varied in the biofilms, and molar ratios did not differ notably among them. Only the *Orange* community showed certain phosphorus deficiency with respect to the carbon content. This biofilm also showed the highest C/N relationship. Both C/N and N/P molar ratios varied in a similar degree, ranging from 7.24 to 17.08 and from 8.97 to 17.68, respectively. The C/P ratio showed the widest variation among biofilms, with high and low values of 106.38 to 211.40, respectively. Some differences were observed concerning the ^{13}C natural abundances among the biofilms (Table 8.4). The lower delta values of -21.3 ‰ and -16.1 ‰ occurred in the *Green* and *White* biofilms, respectively, while relatively enriched delta values of -14.5, -13.3 and -11.7 ‰ were observed in the *Orange*, *Deep Black* and *Black-Striped* biofilms respectively.

Table 8.3. *Relative abundances of different phototrophic microorganisms inhabiting the five biofilms, indicated as follows: +, present; ++, frequent; +++, abundant; –, absent. Biofilms are GR (Green), DB (Deep Black), BS (Black Striped), OR (Orange) and WH (White).*

Taxa	GR	DB	BS	OR	WH
Cyanobacteria					
Oscillatoria sp1. (5-6 µm width)	+	++	+++	++	+
Oscillatoria sp2. (9-10 µm width)	-	-	+	+	-
Phormidium cf. *autumnale*	-	++	-	+	-
Leptolyngbya sp.	-	++	+	+++	-
Bacillariophyceae					
Amphora veneta	-	-	-	-	+
Brachysira minor	-	-	-	+	-
Caloneis bacillum	-	-	-	-	+
Chamaepinnularia gerlachei	++	-	+	+	-
Chamaepinnularia krookiiformis	-	-	-	+	+
Diadesmis arcuata	-	-	-	-	+
Diadesmis comperei	-	+	-	+	+
Eolimna jamesrossensis	-	+	-	-	+
Fragilaria capucina s.l.	+++	+++	++	+++	++
Gomphonema signyensis	-	-	+	-	+
Gomphonema sp.	-	+	-	++	++
Hantzschia amphioxys	-	-	+	-	-
Hantzschia hyperaustralis	-	-	-	-	+
Hippodonta hungarica	-	-	+	-	+
Luticola australoatlantica	-	-	+	+	-
Luticola cohnii	-	-	-	-	+
Muelleria australoatlantica	-	-	-	-	+
Navicula australoshetlandica	-	-	-	+	+
Navicula cremeri	-	+	-	-	-
Navicula gregaria	-	-	-	-	+
Nitzschia acidoclinata	-	+	-	-	-
Nitzschia cf. gracilis	+++	++	+	+	++
Nitzschia frustulum	-	+	+	-	+
Nitzschia homburgiensis	-	-	+	-	+
Nitzschia inconspicua	++	-	-	+	+
Nitzschia paleacea	-	-	+	+	+
Nitzschia perminuta	+	-	-	+	+
Pinnularia borealis scalaris	-	-	-	-	+
Pinnularia microstauron	+	+	-	-	+
Pinnularia subantarctica elongata	-	-	-	-	+
Planothidium delicatulum	-	+	-	+	+
Planothidium frequentissimum	-	-	-	+	+
Planothidium haynaldii	-	-	+	-	-
Planothidium lanceolatum	-	-	+	-	+
Psammothidium metakryophilum	-	-	+	+	+
Sellaphora nana	-	+	-	-	+
Staurosirella pinnata	-	+	-	-	-
Green algae					
Ulothrix sp.	+++	+	-	-	+++
Chrysophyceae					
Ochromonas-like	+++	++	-	+	+++

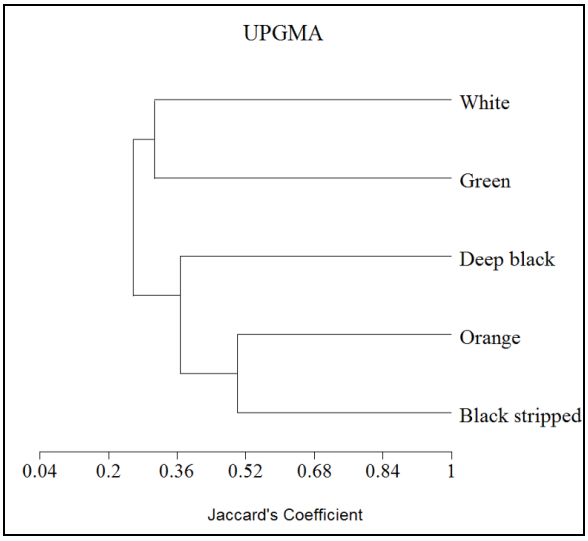

Figure 8.2. *Biofilms cluster of UPGMA linkage using the Euclidean distance. Mainly, the analysis revealed two major groups based on algae assemblages: 1) White and Green and 2) Orange and Black Stripped. Deep Black likely represents a transient assemblage among them.*

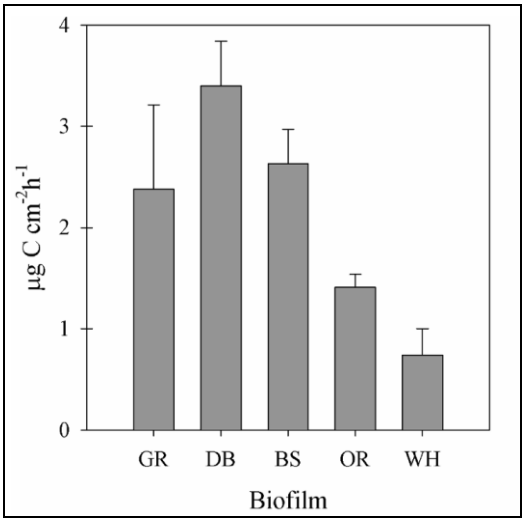

Figure 8.3. *Areal photosynthetic rates of inorganic carbon assimilation measured in the five biofilms. Green (GR), Deep Black (DB), Black-Striped (BS), Orange (OR) and White (WH). Uptake rates were obtained by subtracting values obtained at dark conditions to those obtained under illuminated conditions. Average photosynthetic active radiation (PAR) light during incubations was 250 µE* $m^{-2}s^{-1}$.

Table 8.4. *Elemental composition (Carbon, Nitrogen and Phosphorous) of bulk biomass and natural abundances of ^{13}C in the five biofilms studied.*

	Unities	Green	Deep Black	Black-Striped	Orange	White
C	mg g dw^{-1}	255.21	187.70±50.	208.76±17.8	187.54±25.	38.84±4.5
N	mg g dw^{-1}	36.94±	30.07±6.8	27.28±0.4	12.82±1.9	3.87±0.0
P	mg g dw^{-1}	5.20±0	4.49±0.0	3.42±0.2	2.36±0.4	0.99±0.3
C/N	molar ratio	8.03±0	7.24±0.4	8.92±0.6	17.08±0.2	11.70±1.2
N/P	molar ratio	15.85±	14.82±3.3	17.68±1.3	12.40±3.9	8.97±2.3
C/P	molar ratio	127.94	107.95±29.	158.15±22.5	211.40±65.	106.38±38.
$\delta^{13}C$	‰	-21.30	-13.30	-11.70	-14.50	-16.10

8.3.5. Exopolymeric substances (EPS) distribution in biofilms

The heterogeneity observed among biofilms was also reflected in their exopolymeric substances (EPS; Figure 8.4a). Significantly higher amounts of EPS per dry weight were observed in *Deep Black* biofilms, whereas *Green*, *Black-Striped* and *Orange* biofilms all had similar values. The *White* biofilms showed significantly lower values. Weight ratios for EPS carbohydrate and protein content also varied notably (Fig. 8.4b). A significantly higher proportion of carbohydrates relative to proteins was observed in *Orange*. This ratio was also significantly higher in *White* biofilm with respect to the *Green*, *Deep Black* and *Black-Striped* biofilms, which showed lower ratios. Since the EDTA-extractable carbohydrates comprise the EPS-matrix of biofilms (colloidal fraction) as well as the sheaths of microorganisms (capsular fraction), these values represent the sum of both. Using the calcofluor white probe, a stain for carbohydrates, we identified the fraction of EPS associated with cyanobacterial sheaths (Fig. 8.5), confirming that this fraction differs among the cyanobacterial morphotypes. *Oscillatoria* sp1, which was abundant in cyanobacterial dominated biofilms, was positively stained with calcofluor. It was characterized by a thick polysaccharide layer of carbohydrate inclusions (Fig. 8.5b), likely composed of glycogen. As shown in figure 8.5a, a large number of diatoms were attached to the thricomes of this cyanobacterium. The *Leptolynbya* dominating in the *Orange* biofilm also showed an EPS-rich sheath (Fig. 8.5c), whereas *Oscillatoria* sp2 had a more discrete layer of polysaccharides deposited outside the cell (Fig. 8.5d). By contrast, the *Phormidium* observed in this same biofilm lack fluorescent signals when excited (Fig. 8.5b).

8.3.6. Principal components analysis

A Principal Components Analysis (PCA) was performed to test the weight of different factors explaining the variability observed between biofilms (Fig. 8.6). Components 1 and 2 comprised 79.59 % of total variance. Component 1 accounted for 54.91% of variability and was strongly associated with nutrient status and photosynthetic activity. The ratio between carotenoids and chlorophylls (ratio 480/665 nm) had by contrast a lower weight, as indicated by the small range of the axis score for this variable. In this axis, the *White* biofilm was clearly separated from the rest on the negative side, indicating a close relationship with degradation products and low photosynthetic activity. On the other hand, component 2 accounted for 24.67 % of variability. In this axis, myxoxantophyll and $\partial^{13}C$ had higher positive weights, whereas fucoxanthin had the highest negative loading, demonstrating a close relation between this component and the taxonomic composition of biofilms. Accordingly, biofilms dominated by cyanobacteria (*Deep Black*, *Orange* and *Black-Striped*) were located on the positive side, while *Green* was on the negative side.

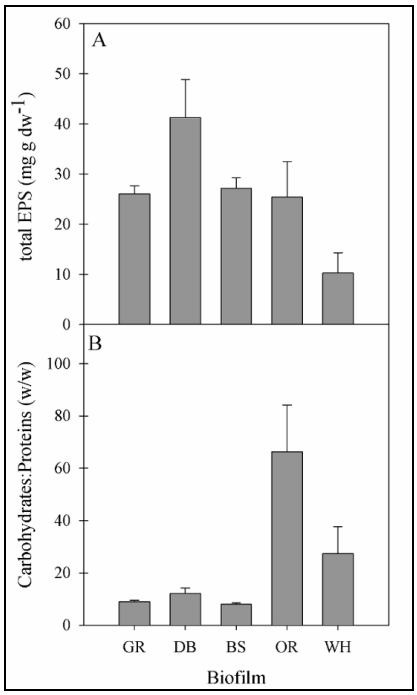

Figure 8.4. *Distribution of exopolymeric substances (EPS) in the biofilms. A) Total amounts of EDTA-extractable EPS in biofilms. B) Weight ratios of carbohydrates versus proteins in EPS extracted from biofilms.*

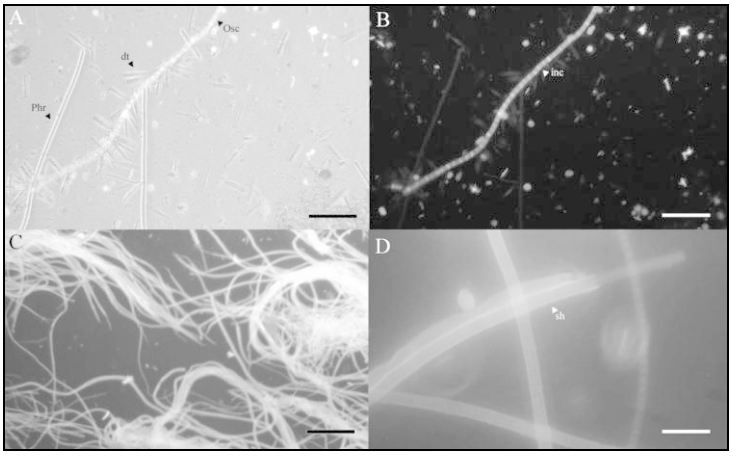

Figure 8.5. *Visible light and epifluorescent microscopy photographs of biofilms stained with Calcofluor White dye showing common cyanobacterial morphotypes found in the biofilms of Peninsula Byers. A) Visible light picture from biofilm showing Phormidium (Phr) and Oscillatoria sp1 (Osc) filaments attached by numerous diatoms (dt). B) Epifluorescence image from same field showing only Oscillatoria filament and the presence of carbohydrate inclusions (inc) in cells. C) Epifluorescent pictures of Leptolynbya from Orange biofilm showing EPS-rich glycocalix sheath (sht). D) Oscillatoria sp2 from Orange biofilm showing a polysaccharides layer deposited outside the cells. Bar indicates 50 µm in all cases.*

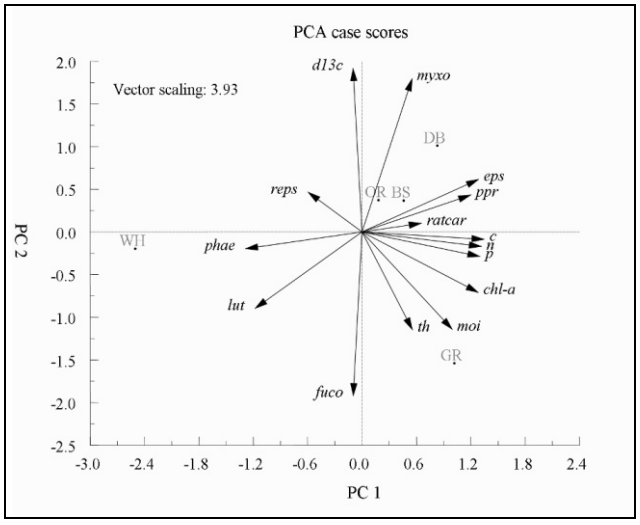

Figure 8.6. *Distribution of biofilms and measured variables plotted by 1st and 2nd components of PCA analysis. Labels are: th: biofilm thickness; c: Carbon; n: nitrogen p: phosphor; eps: total EPS reps: ratio carbohydrates versus proteins in EPS; moi: biofilm moisture; chl-a: Chlorophyll-a per surface; ratcar: ratio 480/665 nm; $d^{13}c$: $\partial^{13}C$ in biofilm; ppr: photosynthetic rates; phae: Phaeophytin-a; fuco: Fucoxanthin; myxo: Myxoxantophyll; lut: Lutein.*

8.4. Discussion

Our findings demonstrate a precise distribution of biofilms along the riverbed. The main structural factor behind the observed spatial heterogeneity of biofilms appears to be the selective stresses exerted by the water regime. As observed in other Antarctic streams, strong currents can limit periphyton growth by scraping and lifting off algal biomass from the stream substrate (ELSTER AND KOMAREK 2004 and articles cited therein). This occurs often when running water contains an elevated load of suspended particles. The central stream channel was densely overgrown by a biofilm dominated by filamentous chlorophytes, whereas those situated in the margins and rivulets, and especially those isolated from continuous water flow, showed assemblages that ranged from a clear dominance of cyanobacteria to those with abundant diatoms. The development of cyanobacteria in the centre of the channel thus appears restricted by a higher current, favouring biofilms composed of chlorophytes that can thrive in more turbulent conditions.

The dominant micro-organisms observed in these biofilms are common in cold environments. For instance, *Phormidium* cf. *autumnale* has been reported in biogeographical studies as frequently observed taxa in Antarctic biotopes (TATON ET AL. 2003). The dominant diatom species, *Fragilaria capucina*, is a common constituent of rivers and brooks on several sub-Antarctic islands, forming often populations of more than 90% of all diatoms present in the rivers (VAN DE VIJVER AND BEYENS 1999). The genus is known for its ability to bloom opportunistically, with elevated growth rates and a rapid colonization capability (DENYS 1988, LOTTER AND BIGLER 2000), features that imply adaptation to dynamic environments such as the stream in this study. Although the amount of aerophilic diatoms was too low to indicate that biofilms dry out completely, they were more common in biofilms subjected to more dryness.

Carbon fixation rates measured in the more active biofilms are comparable to those observed in other localities of the maritime Antarctic region such as King George Island (3.0-3.6 μg C cm^{-2} h^{-1}; HAWES 1993). On the other hand, values obtained for the *Orange* and *White* biofilms fall in the range observed for cyanobacterial communities in the McMurdo Dry Valleys (0.39-2.15 μg C cm^{-2} h^{-1}; VINCENT AND HOWARD-WILLIAMS 1986). In our case, biofilms subjected to high water flow showed higher photosynthetic rates, suggesting that productivity and water current may be coupled. Since different nutrient uptakes can occur due to differences in the water flow velocity (SIMON ET AL. 2004), it is possible to relate this with certain functional aspects of biofilms. For example, as MORRIS AND

MONIER (2003) noted, biofilms tend to have rough surfaces under turbulence or shearing stress, involving a reduction of boundary layer thickness. This results in a net increase of photosynthetic rates due to a facilitation of mass transfer within the biofilm–water interface (CHARACKLIS AND MARSHALL 1990). Hence, a competitive advantage may thus be gained by coping with an elevated water flow.

As mentioned before, both *Orange* and *White* biofilms showed lower areal photosynthetic activity compared to other biofilms. However, for specific photosynthetic activity (i.e., values normalized to Chl-*a* content), *Orange* displayed the highest photosynthetic yield. This difference in the photosynthetic rates (areal versus specific), which was also observed in microbial mats from the same site (see chapter 7), indicates that the phototrophic community of this biofilm is devised to maintain low growth rates, which furthermore could be indicating that is well-adapted to thrive under low nutrient conditions. Further parallelisms occur between the present biofilms and the microbial mats studied in the previous chapter. Thus, higher and comparable photosynthetic rates are observed in the microbial mat located in the stream and in the biofilms less affected by water availability (*Deep Black*, *Green* and *Black-Striped*). On the contrary, the microbial mats from soils and ponds displayed similar photosynthetic rates to the *Orange* biofilm, and all were characterized by a higher drought stress.

Our findings suggest a trade-off between water current (i.e., water renewal) and nutrient availability. Presumably, biofilms establish in the riverbed in the short time period just after thawing, just when the water discharge on the drainage channels are highest. When the present study was carried out (February), flow was low and therefore, stresses associated with full-flowing events were partially relieved, even though stresses related to low nutrient renewal should be high at this time. A physiological study performed in another part of Livingston Island involving carbon and nitrogen dynamics in two benthic communities confirms this hypothesis (DAVEY 1993). In that study, although the author proposed that algal biofilms were not limited by nutrient availability, evoking instead a major role for other physical factors (irradiance and temperature), he found that atomic ratios (N/P) were more balanced in the mat inhabiting the central channel (dominated by chlorophytes) compared to mats on the margins (dominated by cyanobacteria). Our results support those observed along a gradient of water availability by ELSTER (2002). This author stated that in polar environments filamentous cyanobacteria dominate habitats with reduced water availability, probably related to the ability to recover from freezing and/or desiccation.

Although EPS production can also be ascribed to other microbial components of biofilms like diatoms (DE BROUWER AND STAL 2002, CHIOVITTI 2003), bacteria or chlorophytes (KROEN 1984, PAULSEN 1994), in our case, their occurrence seems related to adaptive strategies involving cyanobacteria. For instance, EPS are known to offer adaptive features for coping with dryness. In this sense, the moisture content of the *Orange* biofilm is greater than expected due to its stream location. The EPS matrix provides a constant degree of moisture inside the biofilms, which might explain why this biofilm was even wetter than *Deep Black* biofilm. EPS likely has additional physiological functions. Unlike the other biofilms, whose molar ratios did not show a notable nutritional unbalance, *Orange* biofilms had a clear phosphorus deficiency with respect to its carbon content, as well as higher C/N ratios. As noted before, this nutritional unbalance might be due to limited nutrient renewal as a consequence of limited water availability. This nutrient deficiency could also explain the higher relative content of carbohydrates compared to protein in the EPS composition, since carbohydrates have a much higher content of carbon relative to nitrogen or phosphorus compared to proteins. In addition, although this biofilm does not contain the highest amounts of EPS per dry weight, it has a high EPS content relative to the photosynthetically fixed carbon, meaning a substantial fraction is assigned to the production of carbohydrate-rich EPS. It is plausible since neither nitrogen nor phosphorus, both limited in this biofilm, are incorporated into carbohydrates. Whereas growth is limited when nutrients are in short supply, cells go on taking up carbon, and therefore primary production progresses at all times. This is a physiological strategy that allows cyanobacteria to keep a balanced elemental composition in cells while driving out secondary metabolites with an elevated carbon quota. This mechanism was reported by OTERO AND VINCENZINI (2004) in experiments showing how EPS production in a cyanobacterial strain of the genera *Nostoc* worked like a carbon sink when the C/N relationship in the media was high. The high content of EPS relative to biomass might also allow the biofilm to cope with moisture stress, as suggested above.

The mechanism described for the *Orange* biofilm could also take place in the *Black-Striped* biofilm, though to a lesser extent. In this case, the C/P ratio also indicates a possible phosphorus deficiency. The *Black-Striped* biofilm is located where low nutrient renewal occurs during periods of low water flow. It is unclear, however, since it might rely on the methodological procedure used for measuring EPS. BELLINGER and co-workers (2005) showed that much of the sugar measured in EPS extracts derives from intracellular sources such as an excess of glucan produced during photosynthetic anabolism. Nevertheless, the EDTA-extractable EPS encompasses both colloidal and capsular fractions, meaning that cyanobacterial

sheaths are also obtained during the process of extraction. Therefore, we do not know with certainty what proportion of EPS originates from colloidal or capsular fractions. The occurrence of species with carbohydrate-rich sheaths in the *Orange* biofilm, as demonstrated by staining with calcofluor white (see figure 8.5), indicates that carbohydrate accumulation must occur primarily in the mucilaginous sheath of biofilm cyanobacteria (both *Leptolynbya* and *Oscillatoria*). The $\partial^{13}C$ signatures observed may indicate also differences in biofilm taxonomic compositions and physiological activities related to EPS production. Although both chlorophytes and cyanobacteria use the Calvin cycle for carbon assimilation, and thus generate similar delta values compared to other metabolic pathways (ENGEL ET AL. 2004), carbohydrates tend to become more enriched with the heavy isotope (VAN DONGEN ET AL. 2002). Consequently, if a substantial part of the carbon assimilated in the biofilms dominated by cyanobacteria is derived to the synthesis of polysaccharides (mainly EPS), it is possible then that they display more positive $\delta^{13}C$ signatures compared to biofilms dominated by chlorophytes.

Unlike biofilms dominated by cyanobacteria, those formed by green algae cannot overcome stress factors beyond their range of distribution and thus react differently to desiccation. For example, higher numbers of protozoa and invertebrate fauna in the *White* biofilms (personal observation) indicate significant invertebrate bioturbation. The role of EPS as a cyanobacterial defensive strategy against protozoa grazing has been previously reported (PAJDAK-STÓS ET AL. 2001). Since sheathed cyanobacteria are scarce in the *White* biofilms, this biofilm is likely more disposed to grazing losses. This idea is also supported by the high phaeophithin-*a*/Chl-*a* ratio of this biofilm compared to the other biofilms, indicating the occurrence of much algal detritus (CAMACHO AND DE WIT 2003). These biofilms are likely in a senescence state, as seen in highly productive green algae biofilms experiencing progressive degradation when non-autotrophic biological activities prevail. It might correspond to a more extended distribution of this algal assemblage during ice melting, when stream flow was higher and water covered more of the basin surface. This contrasted with the perennial character of the slow-growing cyanobacterial biofilms, which cannot grow under fast-flow conditions. In summary, we have established how different strategies allow biofilms to mutually progress within a restricted waterfall environment by adjusting their taxonomical composition and functions to particular conditions.

9. Effects of nutrients addition in the structure and function of a microbial mat

9.1. Introduction

Microbial mats are considered appropriate models for studding interactions between microorganisms and the biogeochemical fluxes (STAL AND CAUMETTE 1994, CAMACHO AND DE WIT 2003, BONILLA ET AL. 2005). The ecological implications of further inputs of nutrients in these communities can be explored by means of manipulative experiments. In practice, this allows to assess the carbon fluxes in mats depending of their nutrient status, which would lead to know if mats could be either source or sink of energy in polar ecosystems. Several works focus in the effects of resource availability in microbial mats (PINCKNEY ET AL. 1995a, 1995b, REJMÁNKOVÁ AND KOMÁRKOVÁ 2000; CAMACHO AND DE WIT 2003, BONILLA ET AL. 2005). These studies reveal, in more or less extent, changes in functional and structural characteristics after the nutrients alteration. In Antarctic ecosystems, the scarcity of inorganic nutrients is often the limiting factor for growth; however, at present there are no studies with Antarctic mats such as the mentioned above. To transfer those results to the Antarctic context can be furthermore tentative since the physical control likely disguises underlying factors.

A study performed with two microbial mats in Livingston Island examined the dynamics of carbon and nitrogen, and suggested that these communities were not limited by nutrients (DAVEY 1993). This author attributed a major role to the physical factors (i.e., irradiance and temperature), even so, he found a relationship between the stoichiometric composition of different mats and their position in the riverbed. Likewise, the structure of benthic communities that we observe in Byers depends in part of moisture but also of the nutrient availability (see chapters 7 and 8). Our hypothesis is that the structure of these mats could be regulated in part by biotic interactions such as the resource competition. On the basis of the mechanisms described by the resource competition theory (TILMAN 1982; TILMAN ET AL. 1986), it is expected that an unbalanced availability of nutrients induce a shift of the relative dominance of different organisms competing between them. This responds to the occurrence of different uptake efficiencies and competitive abilities of each organism at each particular nutritional condition. With regards to diatoms and cyanobacteria, which dominate in microbial mats from Byers (see chapter 7), their efficiency to take up nutrients might vary depending of the substrate concentration. Cyanobacteria, for instance, can take a competitive advantage because the capability to obtain nitrogen from N_2 fixation or from storage compounds (e.g. cyanophycin, OBST and STEINBÜCHEL 2006). As well, they are able to accumulate inorganic phosphorus in the form of polyphosphates (CHEVALIER ET AL. 2000, ROESELERS ET AL. 2008).

To test the effects of resource ratio availability in a microbial mat (in our case nitrogen and phosphorus), we chosen the mat with a more equilibrated nutrient composition (i.e., the stream mat described in chapter 7), thus expecting a higher responsiveness to alterations. This mat was submitted to an artificial increment of the ambient inorganic nitrogen and phosphorus concentrations (both separately and jointly). After which, some structural and functional aspects of mat were studied. An increase of precipitation and/or run-off resulting from warming would have immediate effects on the hydrological balance and nutrients fluxes in the ecosystems that these mats inhabit. Our aim was then to test if some postulates of theoretical ecology (TILMAN ET AL. 1986) are also suitable in environments submitted to a strong physical control, thus allowing us to assess the effects derived from a regional climate change.

9.2. Methodology

9.2.1. Experimental setup

The experiment was carried out with one of the mats described in chapter 7 (stream mat). This mat was restricted to the margins of streams located in the south beaches of the region and, differently to the others, was permanently covered by water. For the experiment, several portions of the mat were transferred to PVC containers with an inner surface of 70 cm^2. We proceeded taking care that fragments fitted with the containers walls (Fig. 9.1). The flasks containing mat portions were filled with stream water pre-filtered through GF/F filters and subsequently submitted to different treatments. The four experimental conditions consisted in the increase of natural nitrogen and phosphorus concentrations delivered both separately and jointly (see table 9.1). The combined addition of nitrogen and phosphorus was in a molar ratio of 15. This is according to the Redfield ratio, which is optimal for the growth of benthic microalgae (HILLEBRAND AND SOMMER 1999). During the duration of the experiment (16 days), microcosms were deposited in a shallow stream with continuous flow in such a way that temperature kept between 1.64-2.77 ºC. During the time of the experiment, nutrients were added again at approximately intervals of 3-5 days to prevent exhaustion.

Table 9.1. *Treatments performed in each flask with the stream mat. The conditions assayed consisted in the increase of basal nutrient concentrations of the overlying water. Numeric values indicate this increment. All conditions were assayed by triplicate.*

Code	NH_4NO_3 (µM)	Na_2HPO_4 (µM)
C (control)	0	0
+N	60	0
+P	0	4
+NP	60	4

9.2.2. Measurement of physiological activity and sampling

In order to observe trends in the photosynthetic activity, both profiles of oxygen concentration and photosynthetic oxygen evolution were performed by triplicate in each flask after incubation. Profiles were made as described in section 7.2.2. With the results obtained, both oxygen production and consumption were estimated by observing the oxygen fluxes against depth. These fluxes were calculated according to Fick's first law of diffusion (COHEN AND ROSENBERG 1989) using the following equation:

$$J_z = \phi D_s (dC/dx) \quad \text{(Equation 9.1)}$$

where J_z is the flux through in the layer at depth z, Φ is the porosity of mat, D_s is the diffusion coefficient of oxygen within mat, and dC/dx is the slope of the oxygen profile against deepness at depth x. A negative flux refers to a net downward flux, whereas a positive flux refers to a net upward flux. All microelectrodes measurements were confined to the active layer of the mat (i.e., 0-3 mm).

Once microelectrodes measurements were finished, cores with a diameter of 1.5 cm were collected from each flask to measure the rates of $H^{13}CO_3$, $^{15}NO_3$ and $^{15}NH_4$ uptakes, and N_2 fixation. This was performed following the methodology described in sections 7.2.3, and 7.2.4, however, neither incubations at dark or with DCMU where made in this case. We follow this procedure considering that carbon uptake in this mat was almost entirely performed via oxygenic photosynthesis (see chapter 7). The PAR irradiance during incubations was in average 662 mol photons m^{-2} s^{-1}. More cores were subsequently obtained to perform analyses of photosynthetic pigments (HPLC), stoichiometric composition (carbon, nitrogen, and phosphorus) and EPS content. Results are expressed as the mean and standard deviation of three replicates.

9.2.3. Sampling of overlaying and interstitial water of the mat

The overlaying and interstitial water of the mat were sampled at the beginning of the experiment to assess the basal concentrations of inorganic nutrients (NOx, NH_4, SRP and SRSi). The interstitial water was obtained by applying pressure on untreated fresh cores, thus allowing that pore water came out from the inner parts of the mat. When experiment was finished, the overlaying water of each microcosm was sampled again to observe variations resulting from the experiment. For the conservation and analyses of samples we proceeded as explained in section 2.2.

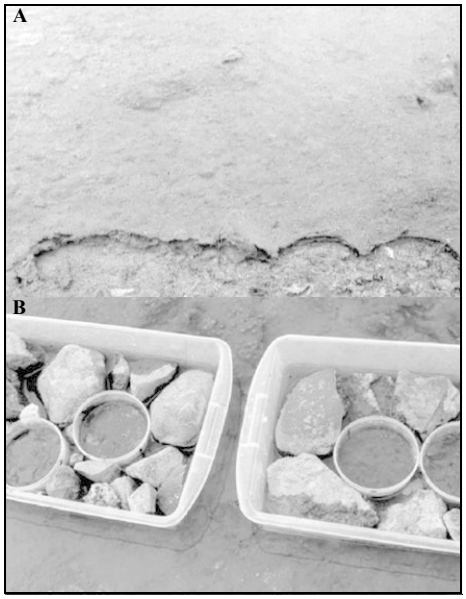

Figura 9.1. *Setting up of the experiment performed with the stream mat. A) Image of the site where samples were picked up. B) The fragments of the mat were introduced on to PVC containers with an inner diameter of 70 cm^2 and then submerged in water running. Samples were prepared taking care that fragments were perfectly fitted in with the containers walls.*

9.2.4. Data analysis

To check if differences observed were statistically significant a one-way ANOVA and post-hoc comparisons were made between treatments. Non-parametric analyses were made when the Levene test for variance was not satisfied. In all cases the statistical significance was delimited to a p-value of 0.05.

9.3. Results

9.3.1. Chemical conditions in the mat before enrichment

In the table 9.2 are shown the ambient concentrations of the major inorganic nutrients both in the overlaying and the interstitial water of the mat before the enrichment. The conductivity and pH in the overlaying water were in average 78.0 $\mu S\ cm^{-1}$ and 6.32 respectively. The concentrations of ammonium (NH_4) and reactive soluble phosphorus (SRP) in the pore water were around 4-fold higher compared to the overlaying water. Nitrate plus nitrite (NO_x) was also higher inside the mat although a little. By contrast, the concentration of the soluble reactive silica (SRSi) in the overlaying water was the double to those measured in the interstitial water.

Table 9.2. *Molar concentrations (µM) of main inorganic nutrients measured in overlay and interstitial water before experiment.*

Nutrient	Surface water	Interstitial water
NH_4	3.22	11.2
NOx	1.71	1.93
SRP	0.487	1.6
SRSi	77.2	37.2

9.3.2. Structural changes in the mat after enrichment

The experimental enrichment resulted in no significant effects on total photosynthetic biomass, measured as the areal chlorophyll-*a* (Chl-*a*) content (Fig. 9.2a). Even so, a significant decrease of the phaeophytin-*a* was observed in the treatment +NP (Fig. 9.2b). Also both +N and +P treatments showed lower phaeophytin-*a* amount compared to controls, although not significantly. Concerning to taxon-specific carotenoids, expressed relatives to Chl-*a*, the phosphorus fertilization induced an increase of myxoxantophyll (specific of cyanobacteria) as observed in figure 9.2c, although differences compared to controls were only significant when it was added alone. On the contrary, in both treatments supplemented with phosphorus (+P and +NP) a slightly but no significant decrease of fucoxanthin (specific of diatoms) occurred (Fig. 9.2d). This structural shift of the photosynthetic community was also reflected in the relationship between fucoxanthin and myxoxantophyll (Fig. 9.3). The lower values of this ratio occurred at treatments involving fertilization with phosphorus (+P and +NP), while the higher were measured in the controls.

The elemental composition of the mat changed moderately due to the experimental fertilization (Fig. 9.4). The carbon and nitrogen content was rather similar between treatments, showing only a little increase in those samples supplemented with phosphorus (+P). Compared to the controls, all treatments that involved any nutrient adding (+N, +P, and +NP) showed an increase of phosphorus content, nevertheless, differences with respect to controls were only significant when both nutrients were added. On the other hand, those treatments including nitrogen amendments (+N and +NP) showed slight increases of the C/N molar ratios (Fig. 9.4), but significant differences were only observed between the treatments +P and +NP. On the other hand, both N/P and C/P molar ratios were lower in those treatments supplemented with nitrogen (+N and +NP) but neither differences were significant. Otherwise, the occurrence of EPS in mats was also confirmed (Fig. 9.5). In agreement with the observed in the previous chapters, the amount of carbohydrates was higher than that of proteins. Among treatments, the fraction of carbohydrates showed a significant increase in the treatment supplemented only with phosphorus compared with the controls and with the treatment fertilized only with nitrogen.

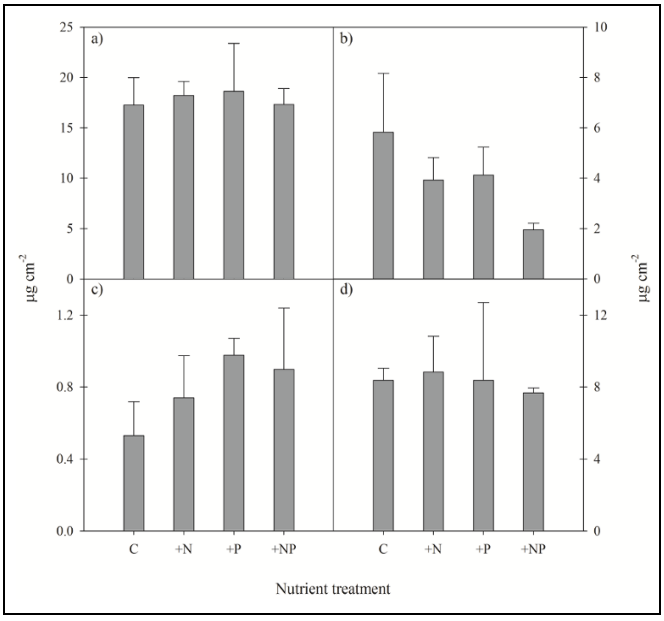

Figure 9.2. *Pigment concentrations in the different experimental conditions at the final of experiment. a) Chlorophyll-a, b) Phaeophytin-a, c) Myxoxantophyll (cyanobacteria biomarker), d) Fucoxanthin (diatoms biomarker). The C refers to the control treatment.*

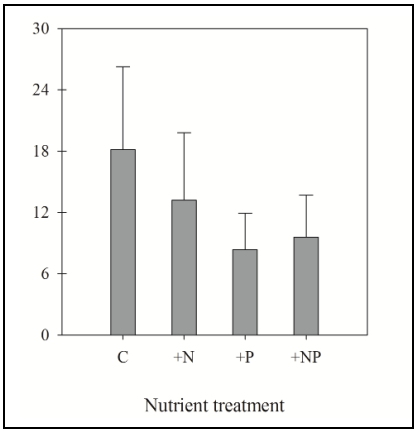

Figura 9.3. *Relative dominance of phototrophic groups in function of nutrient treatments expressed as the weight ratio fucoxanthin(diatoms)/myxoxantophyll(cyanobacteria). The C refers to the control treatment.*

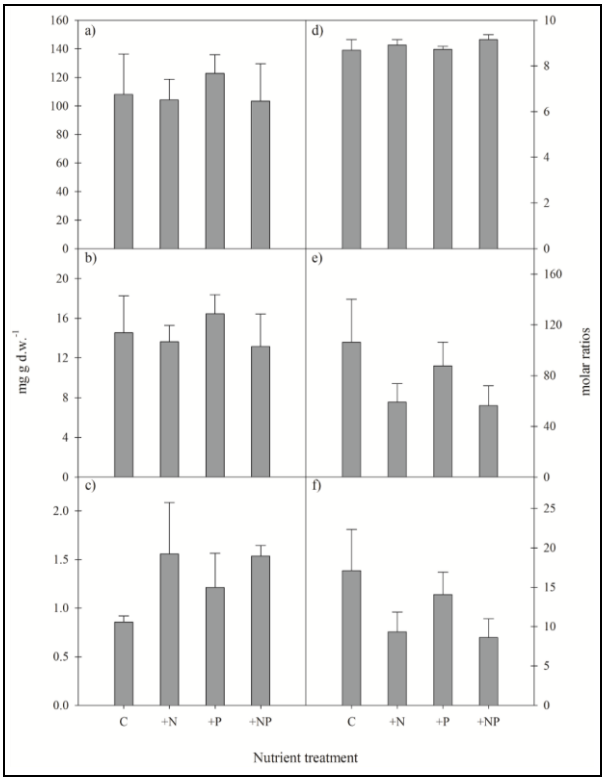

Figura 9.4. *The amounts and stoichiometric relations of major nutrients in the different experimental conditions at the final of the experiment. a) carbon, b)nitrogen, c) phosphorus, d) C/N molar ratio, e) C/P molar ratio, f) N/P molar ratio. The C refers to the control treatment.*

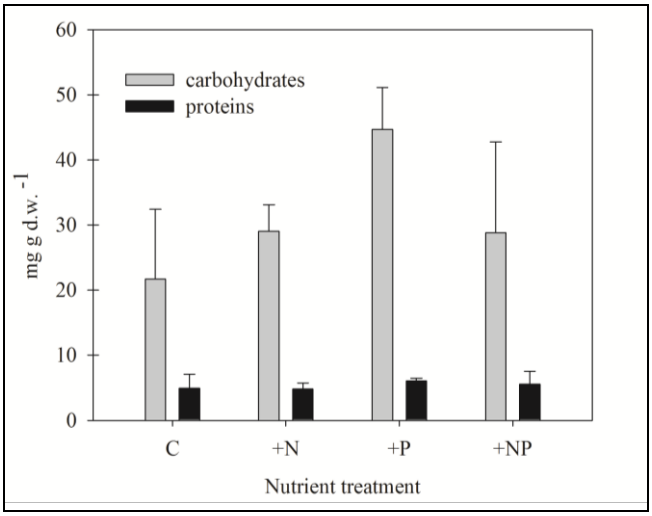

Figure 9.5. *Amounts of the two analyzed types of EPS in different treatments at the final of the experiment.*

Table 9.3. *Post hoc test for differences in pigments, elemental composition, and EPS content between different experimental conditions. ns= not significant.*

	C *vs* +N	**C *vs* +P**	**C *vs* +NP**	**+N *vs* +P**	**+N *vs* +NP**	**+P *vs* +NP**
Chl-a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pheo-a/Chl-a	n.s.	n.s.	0.055	n.s.	0.042	0.036
Myxo/ Chl-a	n.s.	0.022	0.111	n.s.	n.s.	n.s.
Fucox/Chl-a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Carbon	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Phosphorus	n.s.	n.s.	0.036	n.s.	n.s.	n.s.
C/N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C/P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N/P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EPS-carbohydrates	n.s.	0.100	n.s.	0.095	n.s.	n.s.
EPS-proteins	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

9.3.3. Trends in photosynthetic activity after enrichment

The photosynthetic activity in the mat after incubation was assessed following two approaches, both by measuring the inorganic carbon uptake with ^{13}C (Fig. 9.6) and by the light–dark shift technique using microelectrodes (Fig. 9.7). In the former assay, it was detected a stimulation of the photosynthesis when both nutrients were added jointly (+NP), although differences were not significant if they are compared with the other treatments. These other treatments showed similar rates between them, although slightly lower in the treatment enriched only with phosphorus (+P). Concerning to the measures with microelectrodes, the steady-state concentrations of oxygen showed in general an increase with depth, yet, the peaks occurred at different layers depending of treatment. Both in the controls and in the treatment supplemented with phosphorus, the oxygen concentrations peaked between 1.5-2.25 mm. By contrast, the peaks developed shallower, in 1.25 mm and between 0.5-1.25 mm in +N and +NP treatments respectively.

In the figure 9.7 are compiled the profiles of gross photosynthetic rates and the steady-state O_2 concentrations measured in the different treatments. With relation to the gross photosynthesis, an upper maxim production occurred at depths around 0.75 mm in all treatments. Additionally, a deeper peak was observed in the control and in the treatment supplemented with phosphorus. In the later, the underneath peak was even higher than that from the surface. The deepness of the euphotic layer, which is defined as the depth where the photosynthetic rates are still detectable, also differed between treatments. Thus, it was somewhat higher in the control and the treatment supplemented only with phosphorus (around 3 mm) in comparison to the treatments involving nitrogen adding (up to 2.5 mm). On account of this heterogeneity, two depth-integrated rates of gross photosynthetic were obtained by splitting the total profile in two zones, that is, from 0 to 1.5 mm and from 1.5 to 3 mm respectively (Fig. 9.8). In all treatments except in the fertilized only with phosphorus, the photosynthetic rates were higher in the upper layer (0-1.5 mm). These differences were statistically significant in the controls (p-value=0.007) and in the treatment fertilized which both nutrients (p-value=0.018). The vertical fluxes of oxygen were also obtained from the steady-state profiles (Fig. 9.9). In general, all treatments showed an upper layer with a net oxygen upward diffusion and a deep one with a downward flux. The lower fluxes, both upward and downward, were measured in the controls.

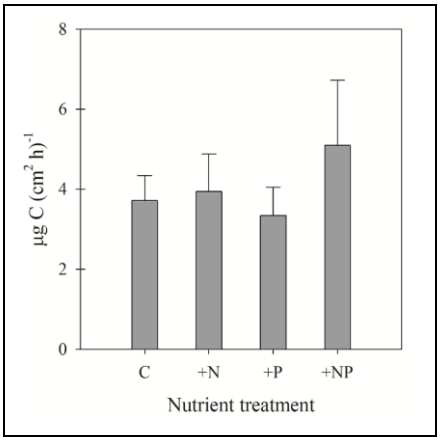

Figure 9.6. *Photosynthetic activity measured as rates of inorganic carbon assimilation in the different treatments at the final of experiment. Incubations were made at illuminated conditions (*Average PAR: 662 mol photons m^{-2} s^{-1}).

9.3.4. Effect of nutrient addition to the nitrogen uptake

The uptake rates of nitrate and ammonium obtained in the different experimental conditions are shown in figure 9.10. In all cases the assimilation of ammonium was significantly higher compared to nitrate ($p<0.001$). For the nitrate uptake, the control and the treatment supplemented with nitrogen showed nearly identical results. The two treatments including the addition of phosphorus (+P and +NP) showed higher rates compared to the formers, in particular that supplemented also with nitrogen, although these differences were not significant. For the ammonium assimilation, higher but not significant rates were observed in those treatments involving any addition of nitrogen (+N and +NP). The nitrogenase activity in the mat after the incubations was in general low as shown in figure 9.11, which differ with the observed in other mats from the site (see chapter 7). With all, some significant differences arose between treatments. Hence, the combined addition of both nutrients caused a decrease in the rates of N_2 fixation compared with the other treatments that remained nearly constant.

The different fractions of dissolved nitrogen, that is, ammonium, nitrate and organic nitrogen (DON), were measured in the overlaying water after the incubations. As expected, those treatments supplemented with nitrogen showed the higher concentrations of total dissolved nitrogen in the overlying water (Fig. 9.12). These pools of nitrogen were mainly composed by nitrate and dissolved organic nitrogen (NOD), being the ammonium the lower fraction. On the other hand, in the

controls and treatments fertilized with phosphorus the DON was clearly the dominant fraction, whereas ammonium was completely depleted.

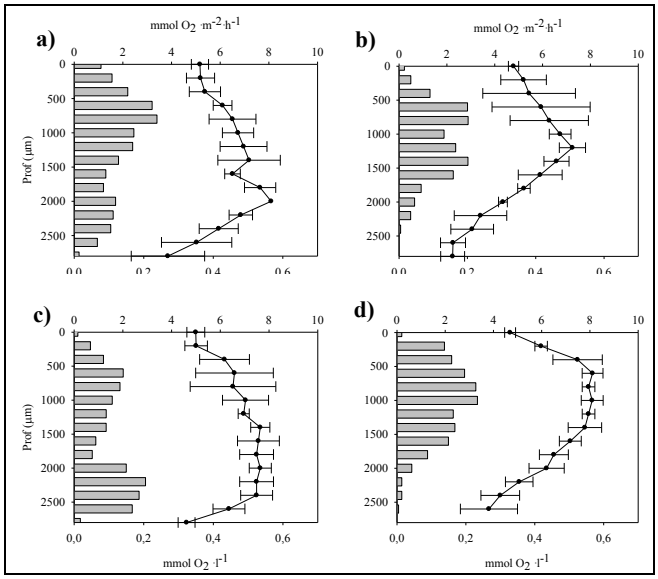

Figura 9.7. *Steady-state oxygen profiles (line, mmol $O_2 \cdot l^{-1}$, mean ± standard deviation from three replicates) and rates of gross photosynthesis at each depth (bars, mmol $O_2 \cdot m^{-2} \cdot h^{-1}$, mean from three replicates) calculated following REVSBECH and JØRGENSEN (1983) in the different treatments: a) Control, b) +60 µM NH_4NO_3, c) + 4 µM Na_2HPO_4 y d) +60 µM NH_4NO_3 +4 µM Na_2HPO_4.*

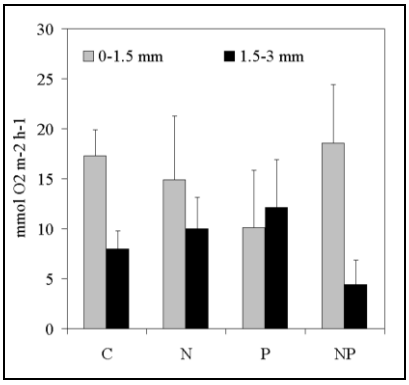

Figura 9.8. *Gross photosynthetic rates measured in the different treatments at the final of the experiment. The two measures showed for each treatment are the result to integrate two different layers in the mat, 0-1.5 mm (grey) and (1.5-3 mm (black). The C refers to the control treatment.*

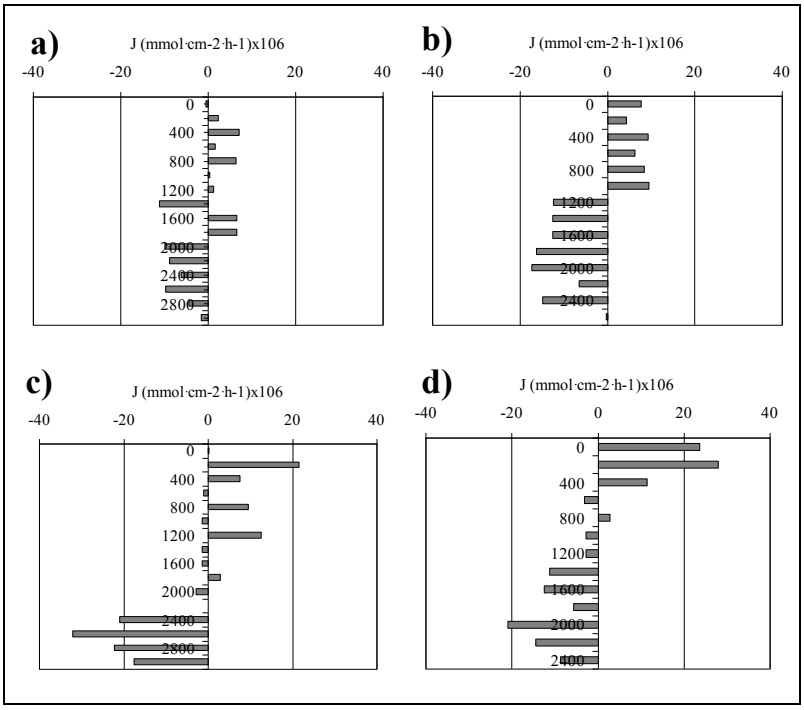

Figura 9.9. *Vertical flux of oxygen obtained in each treatment applying Ficks first law at the final of the experiment. Negative and positive values indicate downward and upward fluxes respectively. Bars represent means from three replicates. The treatments are: a) Control, b) +60 µM NH4NO3, c) + 4 µM Na2HPO4 y d) +60 µM NH4NO3 +4 µM Na2HPO4.*

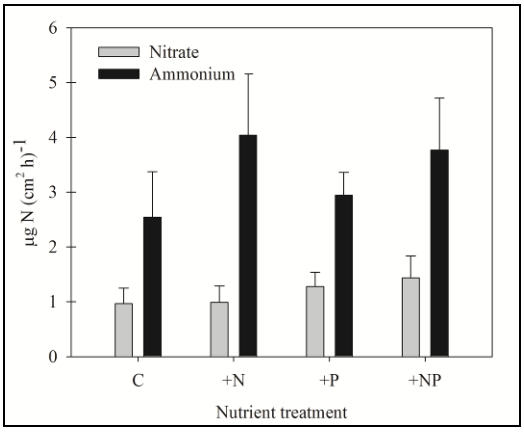

Figura 9.10. *Rates of inorganic nitrogen assimilation on the different treatments at the final of experiment.*

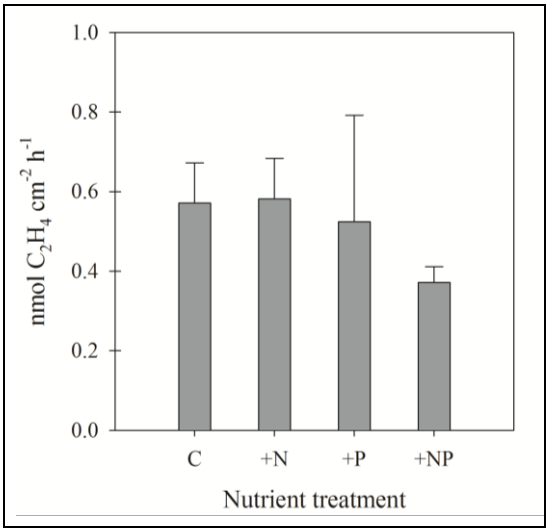

Figura 9.11. *Nitrogenase activity measured as the areal rates of C2H4 production on different treatments at the final of experiment.*

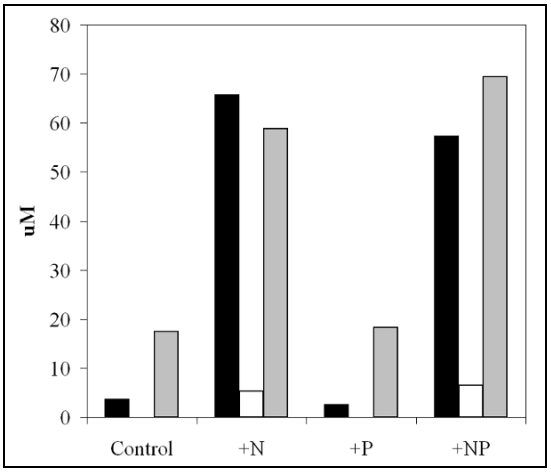

Figura 9.12. *Concentrations of dissolved fractions of nitrogen in the overlaying waters on different treatments at the final of experiment. The bars are NOx (black), NH_4 (white) and dissolved organic nitrogen (NOD; grey).*

9.4. Discussion

The present work is the first attempt to study the effects of inorganic nutrient additions on the structure and function of an Antarctic microbial mat. A similar experimental approach has been only attempted in the Arctic (BONILLA ET AL. 2005). In our case, the ambient concentrations of nutrients show a marked gradient between the overlying and interstitial waters, which agree with the observed in other microbial mats, both in Antarctica (VINCENT ET AL. 1993) and in the Arctic (MUELLER AND VINCENT 2006). It likely implies a higher nutrients availability than the expected by observing the concentrations in the stream. Besides, not only the amounts of nutrients but also their stoichiometry differs between both waters. Though, as indicate BONILLA ET AL. (2005), it does not imply necessarily the absence of nutrient limitation, because the higher accumulation of biomass in the phytobenthos requires a higher amount of nutrients for growth. These chemical gradients occur in the interface of sediment and water in freshwater ecosystems (SCHINDLER ET AL. 1987, HANSSON 1992) and are related with diffusional mechanism. It is due to the resistance to the mass transfer that take place in the diffusive boundary layers (DE BEER AND KÜHL 2001). These gradients increase when the stratification of the water layer covering the mat is more pronounced (MUELLER AND VINCENT 2006). Thus, without turbulence, the mass transport within mat and the overlying water occurs mainly via molecular diffusion. It is not exactly our case, even so, it is possible that the stagnant conditions at which is our mat impedes partially the diffusion. Additionally, the roughness of the mat surface might also hamper the chemical diffusion.

Our experimental manipulation does not generate a detectable accretion of the mat, still, some structural and functional changes occurs. Although the total algal biomass remains unchanged, a decline in the phaeophytin-*a* content is observed in all treatments entailing any fertilization, whatever with nitrogen or phosphorus, particularly if both nutrients are added in combination. Phaeophytin-*a* is a degradation form of the chlorophyll-*a* that is produced when the magnesium linked to the aromatic ring of the molecule is detached. Usually, the relative increment of this derivative is interpreted as the enhancement of senescence processes affecting algal populations (LOUDA ET AL. 2002). In our case, it seems then that the balanced availability of nutrient improves production although it does not involve a net increase of biomass. Furthermore, this relative decrease of the phaeophytin-*a* is lower in those treatments supplemented merely with one nutrient (+N and +P). It indicates that only an equilibrated addition of both nutrients can supply the requirements of phototrophic community, probably by the fact that single addition of

one nutrient would induce limitation by the other. This drop of the phaeophytin-*a*/Chl-*a* quotient has been also observed in a similar experiment and has been interpreted in these terms (CAMACHO AND DE WIT 2003).

Both the combined (+NP) and the single (+P) fertilization with phosphorus cause a weakening of the mat aggregation, that is evident by the fluffy consistency that showed the mat in those cases. It is possible that the higher oxygen evolution produced by the increase of the photosynthesis created an excess of gas bubbles, which caused parts of the mat to lift-off. In part, this idea is held by the observed by SHEPARD AND SUMNER (2010), who studied the influence of light intensity during the growth-dependent aggregation of biofilms. As note these authors, the gas bubbles generated during photosynthesis become more abundant when irradiance increases, thus influencing greatly the morphology of mat. By contrast, they conjecture that a slower growth should result in more organized patterns, which seems to be the opposite of the observed in our mat.

The fertilization with phosphorus promotes in our case the growth of non-heterocystous cyanobacteria relative to diatoms, which is deduced by the increase of myxoxanthophyll relative to fucoxanthin. Interestingly, this increase is higher when phosphorus is added alone rather than when it is added jointly with nitrogen. Also the single addition of phosphorus favored the development of cyanobacteria over diatoms in similar studies (PINCKNEY ET AL. 1995a, CAMACHO AND DE WIT 2003), which supports the belief that some cyanobacteria are worst competitive when phosphorus is in low supply (SMITH 1983). But, as commented above, it is the phosphorus addition rather than the combined supply with nitrogen which induces the higher effects. Probably the pool of nitrogen existing inside the cells, or its recycling inside the mat, is sufficient for covering the cyanobacterial demands. At the end, it would be in agreement with the resource ratio theory that predicts directional changes in the community structure as a consequence of changes in the ratio of the resources supply (TILMAN 1982, TILMAN ET AL. 1986). In this sense, the relative variation of a resource, although its total amount remains unchanged, may produce a selective pressure that causes a redistribution of species dominances. Furthermore, it seems that in our case a counterbalanced redistribution of the biomass of cyanobacteria and diatoms occurs that not produces a perceptible accretion of the mat.

A competitive advantage of cyanobacteria over diatoms could be explained by different mechanisms. When nitrogen is in short supply relative to phosphorus, cyanobacteria obtain the former from the degradation of some rich nitrogen compounds like phycobiliproteins (WYMAN ET AL. 1985) or cyanophycin (OBST and

STEINBÜCHEL 2006). Also differently to diatoms, cyanobacteria can alleviate the nitrogen requirements from the N_2 fixation. The rates of ethylene production are lower in the treatment fertilized with both nutrients, which suggests some regulation of the nitrogenase activity depending of the relative quota of nitrogen and phosphorus. Certainly, the heterocystous cyanobacteria are scarce in this mat (see chapter 7), though they might acquire a higher relevance if the availability of nutrients change significantly.

The observed in the mat on the inorganic carbon uptake agrees with the extended idea that high primary production but also low growth rates are both inherent attributes of microbial mats. Even though the combined addition of nutrients produces an increase of the photosynthetic activity, it is not translated in the increase of the carbon content. In the same way, in neither case the nitrogen content on mat appears to be altered substantially by fertilization. By contrast, all the treatments involving fertilization show some accumulation of phosphorus, thus producing a reduction of the C/P and N/P ratios. Low ratios between the carbon and other major nutrients have been occasionally attributed to a higher capacity of these benthic communities to adsorb and immobilize nutrients (CROSS ET AL. 2005). Still, it is no easy to explain why in our case the higher accumulation of phosphorus occurs when the mat is supplied with nitrogen instead with phosphorus itself.

With relation to the photosynthesis profiles, there is a methodological problem related with the use of oxygen microsensors that may cause underestimations of the photosynthetic rates (WIELAND AND KÜHL 2000). It is because some oxygen evolution can be produced at darkness as a consequence of an enzymatic decomposition of the hydrogen peroxide. Hypothetically, it would explain that the gross photosynthetic rates in the treatment supplied with the two nutrients did not increase despite of the higher carbon uptake. Regardless, our study with microelectrodes shows the primary production in the mat to be allocated differently depending of the nutritional conditions. Particularly when only phosphorus is added, it is observed a development of a deep photosynthetic maximum. Also in this treatment is observed the lower chlorophyll-*a* content relative to carbon. It may occur if a great part of carbon is assigned to the synthesis of EPS, whose amounts are indeed the highest in this treatment. This mechanism has been already suggested in the two previous chapters. In that cases, we proposed that this carbon allocation to EPS might entail a mechanism for dissipating an excess of cellular energy. As we explain in these chapters, an important part of these EPS might derive from cells sheaths, which seems reasonable since cyanobacterial growth is improved in this treatment.

Following an idea exposed by DECHO ET AL. (2003), EPS matrix may indirectly explain the deepening of the photosynthetic maximum observed in the treatment supplied with phosphorus. In the study carried out by these authors with sediments from the Bahamas concluded that the EPS matrix, when occur in sufficient amounts, might reduce the spectral reflectance of the surface. It enhances the forward scattering of light and consequently increases its penetration into the sediment. A direct consequence of that is the deepening of the euphotic layer inside the mat. Additionally, the presence of EPS may prevent the formation of bubbles, which facilitates the oxygen supersaturation (REVSBECH AND WARD 1984). Otherwise, it is known also that EPS contribute to sharp the geochemical gradients (DECHO 2000), thus producing the steep profile observed in this treatment.

Other possibility is the occurrence of a cross-feeding between the production of EPS and bacterial respiration that would explain the higher downward fluxes of oxygen observed at the deepest layers. Photosynthetic activity inside the mat, favoured by the limited diffusion, improves oxygen evolution and the increase of the pH due to the CO_2 consumption. Under these superoxic and alkaline conditions, the production of glycolate and other photosynthates deriving from short-chained carbohydrates can be enhanced (BATESON AND WARD 1988). A study of NOLD AND WARD (1996) performed to know the nature of the metabolites produced during the photosynthetic activity in a thermophilic cyanobacterial mat, indicated that the photosynthate partioning accumulated mostly polyglucose (up to 70% of total incorporated carbon). Interestingly, the accretion of mat in this case was also limited, as the carbon deserved for the synthesis of macromolecules associated to growth (i.e., protein and rRNA) was considerably low. These carbohydrates can be readily incorporated by bacteria, thus avoiding that carbon flushes out from the mat. It is consistent with the idea that both a high activity and slow accretion are inherent characteristics of these microbial communities, which furthermore could be a regular characteristic of cyanobacterial mats despite of environmental conditions.

The NH_4^+ uptake exceeds notably the rates of NO_3^- assimilation in all treatments. It is known that high ambient concentrations of NH_4^+ may inhibit strongly the uptake of NO_3^- by a competence for solute in photosynthetic organisms (DORTCH 1990). Yet, as observed for carbon, no significant increase of the nitrogen content occurs in the mat. It could be related with the increase of the dissolved organic nitrogen (DON) concentrations observed in the overlying water of two treatments involving nitrogen fertilization (+N and +NP). A similar transformation mediated by microbial mats of inorganic nitrogen into dissolved organic nitrogen

has been observed in Dry Valley streams, Taylor Valley, (MOORHEAD ET AL. 1998). Again, it implies that transformation but not a net incorporation of nitrogen occurs in the mat, which underscores the idea that this community is near to a steady equilibrium state.

In summary, the present study improves our knowledge of the functioning of microbial mats in Antarctic ecosystems. Besides, our findings provide an additional confirmation of the Tilman hypothesis (TILMAN 1982; TILMAN ET AL. 1986) in a physical constrained environment, thus demonstrating that different algal groups are specialized on different nutrients equilibriums. The results show both assimilation and loss processes be closely balanced, which translate in none buildup of the mat.

The mat exhibits a slight response to the nutrient amendments, but still, it causes some functional and structural alterations. Likely, extending the time of incubation would provide more significant effects. With all, our results show convincing evidences that, besides to other factors, a shift in the regional dynamic of nutrients might alter the metabolic equilibrium of these microbial communities. In this sense, the slight short-term changes observed in the species composition after fertilization may ensue in important shifts with time. Therefore, although the major repercussion takes place at the physiological level at first time, at long last the effects could lead also into deep changes on the community composition level. Considering so the ubiquity of these mats, they would have the potential to impact significantly on the whole carbon cycle in the site. However, we cannot discard factors other than the nutrients availability regulating the growth of these microbial communities, as discussed in the two previous chapters.

10. General discussion

Byers Peninsula is a good example of a habitat unaffected by anthropic stresses, thus gathering a large number of quite diverse and unaltered aquatic ecosystems. Many medium-to-small freshwater lakes and ponds are scattered throughout the area. They are located in both a plateau at around 90 m a.s.l. and on beaches. The earliest limnological research performed at this site took place during the British Antarctic Survey expeditions in the 1980's (JONES ET AL. 1993, ELLIS-EVANS 1996b, LOPEZ-MARTINEZ ET AL. 1996). These studies stated the site as possibly being the most significant limnological location in the region. However, they have remained unstudied since this time. Furthermore, those seminal studies are devoid of a detailed description of the lakes' food webs functioning and nutrient cycling. Moreover, fundamental knowledge about the role of biotic interactions throughout the region is also sparse. Thus, our aim was to carry on from where these studies ended. To that end, we performed both observational and experimental approaches to examine some critical elements of the ecological theory in an environment that is submitted to a strong physical control.

The thesis focuses on the planktonic and benthic microbial communities inhabiting the lakes and their catchments. The initial part of the thesis studies the limnological characteristics of some lakes and includes an integrated analysis of their planktonic components. These lakes show low biodiversity with a small number of species forming microbial-dominated communities. This is to be expected because of environmental harshness and general low productivity. The lakes' phytoplankton populations mainly comprise diatoms, chrysophytes, picocyanobacteria and chlorophytes. Their biomasses are among the lowest described for maritime Antarctic lakes. As our exploratory analyses reflect (see the PCA's in Chapter 3), the shape of the pelagic food webs appears to be determined by two levels of approximation. The lakes' morphology regulates the relative importance of the pelagic and benthic habitats, whereas nutrient loads determine their productivity. Yet some of the variability observed among the lakes could be a legacy of the landscape. In these lakes, the interactions based on microbial pathways are responsible for a large part of energy transfer. Apart from environmental harshness, the experiments performed in Lake Limnopolar are compelling evidence for the existence of a potential trophic cascade, which could be mediated by the strong top-down regulation of small protozoa by zooplankton. We certainly assume the inherent limitations of the microcosms-based experiments due to confinement and handling effects (CARPENTER 1996, BERTOLO ET AL. 1999). However, these short-term experiences have the overall utility to depict mechanisms that may potentially control the structure of plankton communities.

The lakes in this region are considered to be in a non-equilibrium status. Hence, climax conditions seem to never be achieved by the plankton community. Consequently, biological interactions (e.g., competition, grazing, and facilitation) were originally assumed to play a minor role. However, as we discuss in this thesis, the occurrence of some stabilising forces might partly rule out this idea. In these oligotrophic lakes, proficient trophic pathways are needed to efficiently channel carbon through the food web. Lake Limnopolar houses a few species and apparently exhibits a simple food web. This lake supports plankton in a truncated pelagic food web, where copepod *Boeckella poppei* is the capstone predator. Yet the densities observed during summer for this copepod rather suggest an efficient transfer of energy from lower trophic levels.

Our field observations outline the balance between autotrophic and heterotrophic production in the lakes of Byers, which formally depends on the ratio between organic carbon and major nutrients (HULOT ET AL. 2001). We conjecture that internal autotrophic processes may dominate the metabolism in lakes with higher nutrient loads (see Chapter 3). Conversely, in low productive lakes, where heterotrophic carbon uptake might exceed pelagic primary production, the benthic communities and bottom mosses from the catchment might provide DOC for bacteria supplies. In this sense, it is known that an extreme oligotrophy combined with allochthonous DOC inputs result in a net heterotrophy on the lake's metabolic balance (PÅLSSON ET AL. 2005). These allochthonous inputs should also be more important in small lakes such as Lake Limnopolar. Thus, loadings increase as the ratio between the perimeter and the area, which is higher in small lakes.

Our survey has demonstrated that, as a rule, nitrogen and phosphorus are in short supply in lakes. Manipulative experiments indeed indicate a consistent co-limitation of them both. Still, there are natural nutrient sources in Byers Peninsula resulting in different trophic statuses among lakes. They are in part supplied with nutrients from snow and/or glacial melt waters. In addition, nutrients may originate from the leaching and erosive processes occurring in the inlets. The latter has been described in the catchments of the McMurdo Dry Valleys (GOOSEFF ET AL. 2002). There it is perceived how longer streams have higher concentrations of major ions if compared to shorter ones. These nutrients enter the lakes directly from stream inlets or indirectly by diffuse land runoff. On the other hand, mobilisation of nutrients might also derive from the wind erosion of the catchment material, as observed in the ephemeral wetlands of the Dry Valleys (MOORHEAD 2007). The latter seems probable if we consider the windy nature of the weather on Byers Peninsula. Yet even a higher ingress of nutrients may occur in lakes naturally. This is because of

the occurrence of surrounding animal colonies, whose faeces are washed into drainage systems. Otherwise, our results also indicate that sea spray inputs partly control the mineralogical composition of the lake water. On the other hand, the atmospheric deposition of nutrients is expected to play a more important role in inland lakes because of the poor contribution of inlets and surface run-off.

Interactions between the water column and sediment also appear to be significant in these lakes. Accordingly, we observe how the shallower lakes on Byers Peninsula, independently of faunal eutrophication, tend to be more productive. This can be explained by their greater facility to dilute incoming materials. Moreover, diffusion from sediment likely also regulates the baseline levels of nutrients. This sediment retrieval is observed for instance in Lake Somero. It is a shallow lake situated on the plateau and, therefore, is not affected by marine fauna. This lake also exhibits an abundance of fairy shrimps (*Branchinecta gaini*). This anostraca's activity, together with wind-induced sediment removal, is expected to expand nutrient retrieval from sediments. Nonetheless, when ice covers these lakes, the nutrients pulses mentioned above are deprived. However, the occurrence of a narrow melt-zone around the lakes' edge allows the stream tributary to flow into them.

During the austral summer, these lakes can be highly dynamic and may exhibit significant spatial and temporal heterogeneity. They undergo an active hydrological cycle. Several factors, such as snowmelt, ice cover thaw, rainfall, surface water evaporation and permafrost melting, interact during this period. For instance, water circulation is very different if the lake is ice-covered or not because wind-driven mixing is impeded. Although frozen, the persistence of a temperature-driven turnover is observed which produces advecting water movements. The main heat source at this time is penetrating solar energy, which produces a density-stratified water column. This implies that the water column becomes inversely stratified, thus acting as a solar energy trap. These convective layers might allow some nutrients to diffuse through the water column. A climate warming could notably affect these patterns, which are observed when modifying the seasonal heat budget of lakes.

Ice cover timing also affects phytoplankton community dynamics. It is observed, for instance, in the consecutive study years in Lake Limnopolar, which differed in ice and snow cover. In the summer of 2003-04, the lake remained largely ice-covered as a result of a colder, snowy winter. Greater dissimilarities took place during this year compared to preceding ones. Plankton community structure differed considerably from one period to another. Greater algal and bacterial abundances on

surface layers occurred at the onset of ice melting due to increased nutrients and light availability. Moreover, our results also indicate a certain species-specific preference of light quantity, quality, or both. When ice seals off the water column from stirring wind, some degree of stratification occurs. In this period, although not abundant, picocyanobacteria proliferate at deep layers. They specifically excite the phycobilin pigments by green light, which penetrates most efficiently through the ice (VINCENT AND LAYBOURN-PARRY 2008). Conversely, other algal groups, such as diatoms, show a rather uniform distribution in the water column. However it is more likely that under-ice penetrative convection keeps these pennate diatoms in suspension. Thus, diatoms and loricate chrysophytes not only dominate, but also increase more rapidly by coinciding with higher water column turbulence. Some authors have proposed, however, that this is a valid explanation only when strong currents occur on the days preceding the break-up of the ice cover; in contrast, mechanism(s) other than penetrative convection must keep cells floating before this period (VEHMAA AND SALONEN 2009). As mentioned above, the picocyanobacteria in surface waters typically fall in the low range of 100-200 cells ml^{-1}. However, it is also certain that higher abundances (10-100-fold) may occur at sub-surface depths, as observed in Lake Limnopolar in the summer of 2003-04. The regular low abundances of these picocyanobacteria suggest that protozoa benefit mainly from bacterioplankton by their carbon demands.

Similar events during the convective mixing in Lake Baikal in relation to algal succession have been observed (KELLEY 1997). The efficiency of algal cells' suspension seems to be a function of the quotient between the sinking rate and the fluid's updraft speed. For this author, the mixed-layer thickness partly determines this phenomenon. Interestingly, he proposes changes of light transmission through the ice as an explanation for the inter-annual variations observed on algal blooms. We conjecture that the productivity in our lake during summer relies on the previous winter's weather, precisely when the ice cap is formed. It is interesting to note that the latter is an example of a lagged response, which likely has deeper effects on ecosystem functioning than other immediate causalities. In our case, for instance, it is evident that snow precipitation isolates the underlying ice, thus impeding not only the passage of sunlight, but also heat losses from the lake. Greater snow precipitation has been the argument used to explain the ice-out delay observed in Byers lakes when compared to the Signy Island ones (JONES ET AL. 1993). Apart from these seasonal dynamics, lake-to-lake variability in the nutrient status triggers a consistent variation in the plankton community structure. Our results reveal total phosphorus and N/P ratio as the sensitive indicators of picoplankters' relative dominance. Also, the increase of nutrients in the lakes favours the dominance of

chlorophytes over chrysophytes and diatoms. On the other hand, the nutrients recycling in the pelagic zone could prove crucial to support plankton growth. Hence, copepods' potential capability to regenerate resources availability is put forward in our experiments. Here we observe that zooplankton has an influence on microbial plankton, not only directly by the grazing control, but also via nutrient regeneration through pellets excretion.

In addition to this leakage of faecal pellets, our results indicate that zooplankton also increases nutrients turnover via the "sloppy feeding" phenomenon. This refers to a nutrient release which originates from ungrazed plankton. It occurs when the food transfer between two organisms is inefficient, so that it become higher when the prey is larger in relation to its predator. Particularly in marine copepods, the amount of detritus produced in this way appears to be quantitatively important when the copepod/prey size ratio is below 55 (MØLLER 2005). It is noteworthy that it implies an underestimation of the ingestion rates measured for copepods when exclusively deduced from grazing experiments. By tracing heavy nitrogen (^{15}N), it has also been observed in marine copepods that nitrogen release exceeds body accumulation rates (HASEGAWA ET AL. 2002), thus provoking a cut-off in the food chain between phytoplankton and carnivores. All in all, this is not our case since copepods are the top predators in Byers lakes.

Other mechanisms also result in nitrogen release. In Monterey Bay (California) for instance, BRONK AND WARD (1999) observed that a combination of grazing and a more physiologically stressed phytoplankton led to a greater release of dissolved organic nitrogen (DON). On the other hand, nitrogen release may also occur during bacterivory, as observed by GRUBER ET AL. (2006) with pure bacterial cultures subjected to predation. These authors associated this with the occurrence of higher C/N ratios in protozoa if compared to bacteria. Although our data are insufficient to test this idea, it may be related with the high DON concentrations observed in Experiment II (see Chapter 6) which coincided with the greater bacteriovorus abundances. Occasionally, Byers lakes show somewhat elevated ammonium concentrations in relation to the total combined nitrogen. Generally, ammonium is a biological by-product that originates during heterotrophic metabolism. Our experiments demonstrate that part of this ammonium might result from copepods' activity. Assumedly, stoichiometry should derive mainly from geochemical processes in highly constrained environments. BARRETT ET AL. (2007) instead demonstrated the occurrence of a biotic processing of the dissolved nutrients in a stream in the McMurdo Dry Valleys. These authors showed how both microbial and metazoan biotas, in accordance with their stoichiometry constrictions, are able

to affect the chemical composition of the surrounding environment. According to our observations, this scenario might occur on Byers Peninsula. Nevertheless, further studies are required to know how much of this remineralization of nutrients supports the lakes' productivity.

Interestingly, a downward loss of nutrients from pelagic compartments has been related with shifts in the phytoplankton size structure (WEHR ET AL. 1994). These authors found that a higher domain of picoplankters involved lower sedimentation rates of particulate phosphorus. In these cases, the pelagic nutrient turnover was likely enhanced, thus reducing the accumulation in the lake's sediment. In our case, the sediment sequestration of nutrients in more productive lakes was probably not significant due to their shallowness. Conversely, in the deeper lakes, where phosphorus supposedly remains immobilised longer in sediments, the strategies adopted to increase pelagic retention and turnover (involving both heterotrophic and autotrophic pathways) should be favoured. Accordingly, the turnover of organic matter and the nutrients release in Byers lakes must be rapid enough to maintain pelagic production. As mentioned above, an exponential increase of phytoplankton production was observed in Lake Limnopolar in summer 2003-04, following a gradual increase of light availability. However, not all the species evolve equally. Some dynamics appears to be regulated by episodic events. In this sense, R-strategists seem to dominate phytoplankton during these periods, which are rapidly growing species that require turbulent conditions to thrive in (*sensu* REYNOLDS 1984). Probably, nutrients are rapidly incorporated into algal, which leads to a large export of biogenic carbon. In contrast during stable periods when small phytoplankton dominates (which could be considered S-strategists), organic matter recycling is higher and sink losses are smaller. In any case, these dynamics do not fit a conventional scheme of community succession given the inter-annual variability of climate at the site, which brings about dynamics that are not totally repetitive and predictable.

Both the bottom-up and top-down forces are not mutually excluded and can act alternatively or simultaneously. For this reason, they are sometimes difficult to separate in practice. Accordingly, we were not able to establish if the patterns of natural abundances and bacterial size distribution observed in Lake Limnoplar were controlled primarily by bottom-up or top-down forces. According to GASOL ET AL. (2002), bacterivory exerts more control on bacterial population dynamics in oligotrophic systems than in more productive environments. With bacteria, it is possible that top-down forces play an important structuring role. In our case, not only grazing pressure, but also nutrient translocation exerted by copepods operate in

combination. For autotrophic picoplankters (APP), it is evident that top-down mechanisms may outline populations; however, our results mostly indicate that they are resource-limited.

Our findings suggest that algae out-compete bacteria when phosphorus concentrations are low. This idea relies in part on the relationship observed between DOC and Chl-a concentrations. It is known that phytoplankton, mainly the larger type, is a poor competitor for nutrients uptake if compared to bacteria when the latter are independent of phytoplankton-produced energy (DRAKARE ET AL. 2003). For instance, higher uptake rates of phosphorus in bacteria compared to phytoplankton have been observed when there is a non-comensalistic relationship between them (JANSSON 1993b); that is, when bacteria are subsidised by other sources (glucose in this case) besides the carbon released by algae. Another microcosm study indicates that bacteria are superior in terms of P uptake, but that have a poor ability to retain it, which would promote a coexistence with algae (VADSTEIN ET AL. 2003). Interestingly, these authors overturn the traditional view by showing algal exudates as the primary DOC source for bacteria in pelagic systems. Instead, they indicate that grazers (rotifers) were the main source of DOC.

It is possible that the affinity for nutrients varies for different algal species. For this very reason, the aforementioned experimental studies of JANSSON (1993b) could not be totally analogous to our observations. It must also be noted that the experiments performed by JANSSON were conducted without nitrogen scarcity, which contrasts with that observed in most lakes from Byers. In our case, additional studies are be needed to better understand which mechanisms, if they occur, facilitate the pelagic community to act as a sink for limited nutrients. It is possible that some amounts of dissolved organic P occur in Byers lakes, mostly in those presenting a high trophic status. If so, the competitive advantage of either algae or bacteria could be also modulated by their capability to regenerate this additional source of P. For instance, in perennially ice-covered lakes of the Taylor Valley, a significant enzymatic activity of alkaline phosphatase has been observed in the size fractionation of bacteria and algae (DORE AND PRISCU 2001). Noteworthily, these authors suggest that the differences observed between lakes as regards phytoplankton P deficiency may not be due to differences in external P fluxes, but to differences in internal phosphorus cycling. It is likely that a quantification of these enzymes would provide a better evaluation of the nutrient deficiency in our lakes than merely quantifying nutrients concentrations.

Furthermore, as discussed below, nutrients retention in the pelagic compartment could also be favoured if trophic interactions involve a microbial loop.

Further experiments performed by JANSSON AND CO-WORKERS (1996) suggest a greater incidence of mixotrophy as a mechanism that resolves this nutrient unbalance. Inherently, it implies a more complex interaction between bacteria and algae than expected. As these authors explain, this mechanism allows the coexistence of P-limited bacteria and N-limited phytoplankton. It is a scheme that advocates for a new paradigm based on the parity between N and P control of phytoplankton production in lakes (LEWIS AND WURTSBAUGH 2008). Certainly, P usually provides a sound prediction for algal biomass; however, in unproductive systems like those from Byers, a co-limitation seems more probable. Besides, unlike direct human influences, atmospheric sources of nitrogen are expected to be low. In this sense, a regular occurrence of nitrogen-regulated production in the Patagonian lakes has been recently proposed (DIAZA ET AL. 2007).

Mixotrophy has been reported as a habitual strategy in the plankton from Antarctic lakes (BELL AND LAYBOURN-PARRY 1999b, 2003). We failed to learn its actual incidence in our lakes in terms of carbon metabolisation when compared to the obligate autotrophy. In line with this, the occurrence of potentially mixotrophic species does not necessarily mean that they are benefitting from this condition. In any case, a large incidence of phagotrophic flagellates (most of them assignable as chysophytes) is hinted at given the observed increase of the relative amounts of Fucoxanthin (w/w Chl-a) in parallel to the N/P molar ratio in Lake Limnopolar. Besides, we must also bear in mind that some of these mixotrophs' own enzymatic activity in the cell surface because it allows them to assimilate DOC (STOECKER AND GUSTAFSON 2003). In any case, flagellate grazing impact estimates made by us using fluorescent tracers suggest a minor role of mixotrophy in Lake Limnopolar compared with other Antarctic lakes (BELL AND LAYBOURN-PARRY 2003), at least at the time when our experiments were conducted. All in all, it must be considered that some of these flagellates could feed only temporarily or likely prefer other food items than our particles. In fact, mixotrophy is a facultative behaviour that depends on environmental conditions to remain active. So, it is possible that mixotrophy either plays a minor role in Byers lakes during summer or constitutes a strategy to sustain vegetative populations during winter (BELL AND LAYBOURN-PARRY 2003). In any case, this idea is based on incomplete data and must therefore be regarded as preliminary.

The DOC concentrations measured in the more oligotrophic lakes on Peninsula Byers fall in the 1 mg L^{-1} range. The source of allochthonous inputs are the benthic communities that flourish in stream inlets and the surrounding areas of lakes (i.e., filamentous algae and cyanobacterial-dominated microbial mats). Some

of the microbial mats that we have studied are located in the catchment area of oligotrophic lakes of the central plateau. A net nutrients release from these communities might result from the balance between erosional and depositional processes. In this sense, the experiment performed with the stream mat demonstrates a notable DON efflux (see Chapter 9). This great potential to transform mineral nitrogen into organic forms has also been observed in other microbial mats from Antarctica (MOORHEAD ET AL 1998). It is possible, therefore, that the nutrients inputs in lakes originate predominantly from the ice melting in early summer, although a relatively higher nitrate enrichment persists throughout the season. This terrestrial support of lake secondary production might partially explain the progressive increase in the N/P ratios observed in Lake Limnpolar in the summer of 2003-04. Indeed, extra nitrogen supply probably derives from the activity of the nitrogen-fixing cyanobacteria present on these mats. We failed to discover, however, if the refractivity of this DOC is low enough to benefit bacteria. In any case, it has been proposed that the bioreactivity of DOC and its N content are both correlated parameters (BENNER 2003).

As regards microbial mats, their structural analysis indicates that they face stressors, such as UV radiation, lack of moisture or low nutrient availability. Hence, their microbial composition implies a habitat-linked heterogeneity. Thus, we observe the same species occurring across differing habitats in their environmental constraints, although they differ in terms of their relative dominances. The co-occurrence of diatoms and cyanobacteria is a regular characteristic of these benthic communities in different Antarctic locations (FUMANTI ET AL. 1997, VINOCUR AND PIZARRO 2000, SABBE ET AL. 2004, SUTHERLAND 2009). Among the mats studied in Byers, stream mat exhibits a relatively higher diatoms domain. This mat was located on the edge of streams and was therefore subjected to certain flow stress. In contrast, those mats located on moist soil and at the bottoms of shallow ponds were dominated by cyanobacteria throughout their vertical profiles. The occurrence of a spatial segregation of different communities' types comes over even more evidently in the study conducted with biofilms (see Chapter 8). Here, communities also consist mainly in cyanobacteria and diatoms, except the Green biofilm, which was dominated by green algae. In this case, stream hydrodynamics determines the biofilms allocation. Biofilms show a patch distribution with a considerable level of spatial heterogeneity. For instance, functionally competent biofilms of chlorophytes are restricted to the central stream channel, which likely indicates adaptation to faster flow events. On the contrary, the communities dominated by cyanobacteria appear under a wide range of environmental conditions. The fast flow of the central channel is apparently unsuitable for cyanobacterial growth, although they were able

to thrive at partially submersed sites, or when even exposed to the higher drought stress. These observations demonstrate that much of the compositional variability observed in these biofilms is due to environmental heterogeneity, which temporarily arises in ecosystems from the maritime Antarctica. The physical rather than the chemical characteristics have been seen to determine the distribution of benthic communities in those streams feeding Lake Fryxell in the Taylor Valley (MCKNIGHT ET AL. 1998), which somewhat agrees with our findings with biofilms. Contrastingly, with the case of microbial mats, our experiments suggest that a competitive replacement of diatoms with cyanobacteria occurs following phosphorus addition. It is possible, however, that the intracellular nutrient concentration regulates this inter-specific competition rather than an external supply. As VINCENT (2000a) noted, fast growth would not be the way by means which cyanobacteria out-compete other organisms in these ecosystems.

Carotenoid pigments have proved to be useful biomarkers to discern which oxygenic phototrophs dominate microbial mats; however, they may also vary depending on functional aspects. Both carotenoids and scytonemin, which occur in the mats from Byers, perform a photo-protective role, including reactions against photo-oxidation. These photo-protective substances protect mats from UV-damage, but reduce mats' photochemical efficiency because light-protection and light utilisation are mutually exclusive strategies. The use of microsensors allowed us to measure the photosynthetic rates without severely disrupting mats. Greater phototrophic activity is restricted to the upper layers of these mats, although it is not absolutely superficial given the occurrence of these accessory pigments. In particular, oxygen profiles present a typical shape with concentrations on the surface which are at equilibrium with the overlying water and increscent with depth until supersaturation in the first few millimetres. It is quite likely that the net balance between photosynthesis and respiration comes close to zero in these mats, as observed in those from the McMurdo Dry Valleys (HAWES AND HOWARD-WILLIAMS 1998). However, they maintain important populations because they suffer minimal losses because the paucity of grazing and the slow decomposition rates.

Furthermore, it must be evaluated if these microbial mats increase the lakes' trophic complexity. The experiment performed with stream mats (see Chapter 9) shows, for instance, a notable DON efflux, which can be an important source of DOM for overlaying water. Another nutrients source deriving from these mats is extracellular polymeric substances (EPS). The exudation of these EPS contributes to sediment stability, but also protects microorganisms from desiccation or grazing by protozoa. Nevertheless, the EPS that we recovered from mats can partly result from

unbalanced growth. EPS consist largely in polysaccharides, and the capsular fraction of these EPS (i.e., sheaths of cyanobacteria) is a structural cell component. Conversely, the colloidal fraction is exuded to the environment. As we discussed in previous chapters, the latter probably originates from a mechanism to dissipate excess carbon.

These organic substances would serve as an energy and nutrient source for the heterotrophic metabolism in lakes. The CDOM characterisation performed in Lake Limnopolar demonstrates the occurrence of chromophoric organic carbon. The macromolecules that exude the phototrophic organisms of mats can be degraded to mono and dimeric unities through the exoenzimatic activity of bacterial populations. These products can be easily reabsorbed by mats and reused then as a carbon source for heterotrophs. However, some authors argue the co-occurrence of water-soluble compounds such as EPS and oligosaccharide mycosporine-like amino acids, which can easily dissolve in overlying water (MUELLER AND VINCENT 2006). The significant amounts of EPS that we found in mats support this hypothesis, although we certainly cannot prove or disprove this theory based on our data. Besides, marine copepods are also known to excrete fluorescent DOM (URBAN-RICH ET AL. 2006). Moreover, as observed in other Antarctic lakes (SÄWSTRÖM ET AL. 2007a, 2007b, 2008, HERBEI ET AL. 2010), an additional source of DOC may originate from viral activity. Additionally, important DOC sources are also likely the benthic mosses developing at the bottom of lakes. These aquatic mosses grow in the basins of lakes deep enough to avoid excessive sediment removal, and are furthermore favoured by the water column's ultra-oligotrophic conditions, probably a major requirement for aquatic mosses growth (WAGNER AND SEPPELT 2006).

The experiments performed in Lake Limnopolar reveal the existence of a trophic cascade in the microbial pelagic food web, which potentially imposes biotic control on the microbial community. We propose a conceptual model to explain the pelagic food web functioning (Fig. 10.1). To define this model, we studied the influence of size-selective and density-dependent predation exerted by *B.poppei* on the dynamics of the microbial pelagic community. Our results agree with the literature and ascertain that this copepod is an omnivore that feeds on both phytoplankton and microzooplankton. Our outcomes furthermore prove that it occurs simultaneously and during different life-history stages. Hence, copepods feed selectively on size, thus preferring moderately large preys (mean: 10 μm) to smaller ones. In agreement with this, both experiments and field observations revealed how ciliates of around this size are scarcer with the presence of *B.poppei*. The experiments conducted with graded copepods densities even show a sharp decline of

ciliates. One scenario that is consistent with this idea is that the copepods in Lake Limnopolar likely have a low impact on photosynthetic plankton sizing of <0.2 µm and 20 µm; that is, picophytoplankton and microphytoplankton, respectively. For different reasons, both are not edible for direct consumption. Conversely, copepods feed more efficiently on particles within the range between these sizes. Assumedly, when an unfavourable phytoplankton size structure dominates in the lake, it is likely that copepods mainly use protozooplankton (nanoflagellates and ciliates) as main food resource. The direct consequence would be the imposition of a top-down control on planktonic communities mediated by a strong top-down regulation of protozoa populations, which indirectly benefits pico-sized organisms (both autotrophic and heterotrophic).

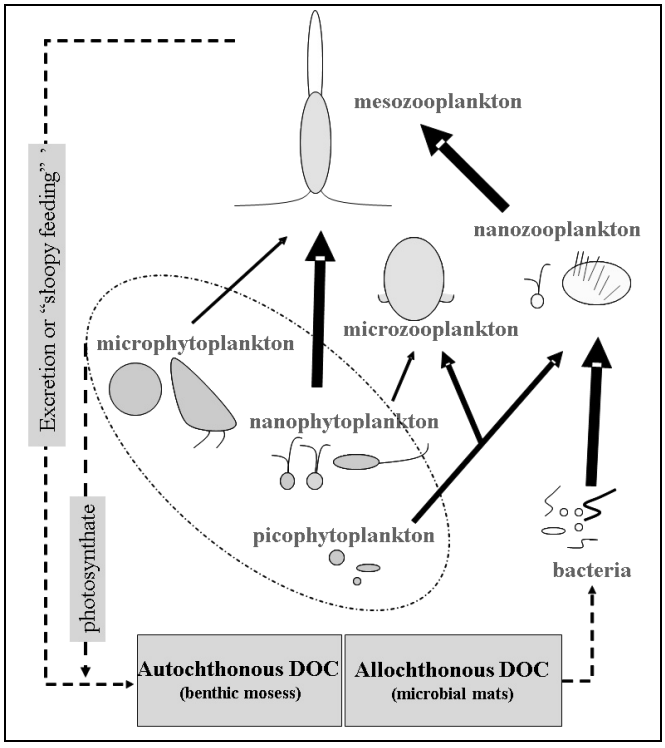

Figure 10.1. *Conceptual model of the pelagic food web functioning in Lake Limnopolar. Arrows thickness indicates the intensity of the interactions.*

We have experimentally demonstrated that the phytoplankton in Lake Limnopolar exhibits different levels of resistance to be grazed by copepods. This

results in a shift in the algal community composition under different grazing regimes. Nutrient uptake is expected to be higher in small cells because of their higher surface to volume ratio, which confers a competitive advantage when nutrients are scarce. However under high nutrient regimes, large phytoplankton out-compete small ones because of the competitive advantages deriving from its larger size, particularly predation avoidance. Here we may discuss if this top-down control can trigger a scenario in which edible species are replaced with inedible species when zooplankton grazing rates are high in the lake. Certainly, this is potentially evidenced in our experiments, but the water column's physical characteristics likely produce rapid changes in phytoplankton species composition. The strength of the top-down interactions depends on the intensity of the trophic linkage between organisms. Thus, the trophic cascade is expected to be more easily promoted in the absence of important cut-offs in plankton size spectra. Some of these cut-offs might occur in Lake Limnopolar, as suggested by the gaps observed in the body-size spectra obtained in the lake (see Chapter 5). As we discuss here, they are partly imposed by physical factors.

It is known that *B.poppei* is able to change habitat use between pelagic and benthic (IZAGUIRRE ET AL. 2003). Its distribution in the water column in the summer of 2003-04 in Lake Limnopolar was vertically segregated, and was mainly nektobenthic in the case of adults stages. Yet in our case, it demonstrates a slight tendency to migrate. Our experiments in Lake Limnopolar also reveal differences among the copepods stages in relation to their prey size preferences. Interestingly, this resource partitioning could determine a different relocation of the copepods in the water column. It is probably the vertical migration phenomenon which provides a niche separation for the different copepods stages. In any case, it seems the water column's physical instability may imply a lack of consistent stratification and mix seasonal patterns. In addition to genetic factors, there should be a facultative component regulating the vertical distribution of copepods. As we argue in Chapter 5, these factors can be light, temperature, and/or food availability. Vertical movements of zooplankton imply considerable energy expense. When considering the absence of external forces (i.e., grazing or UV damage), it leads us to speculate the occurrence of a 'biological pump' mechanism, as suggested in Chapter 5. It is possible then that this migratory behaviour can be associated with a feeding rhythm where light/dark periods alternate. In any case, the data that we provide are possibly anecdotal observations. In this sense, more observations should be made which cover periods with higher copepods densities or when the lake is devoid of its ice cover, which would maybe render more conclusive results. Certainly, our knowledge of the life cycle of *B.poppei* in Lake Limnopolar is restricted to the summer period.

Probably, this copepod maintains high populations, especially when considering that lakes are not wholly frozen during winter and that water remains in a liquid form below the ice cap. In which case, the aforementioned behavioural plasticity might lead to resources to relocate from the pelagic to the benthic habitat.

Our carbon isotopic fractionation data also suggest the occurrence of a strong interaction between the microbial pelagic food web and copepods. Other authors who have studied the feeding habits of *B.poppei* in Antarctica also found that this copepod benefits from pelagic resources (BUTLER ET AL. 2005). However, they did not rule out its potential for benthic browsing if phytoplankton is sparse. Indeed, in a similar approach conducted in a high-latitude oligotrophic lake, KARLSSON AND SÄWSTRÖM (2009) showed that the isotopic signatures of the copepods they presented could be indirectly supported by benthic production during winter, when allochthonous inputs of organic matter and pelagic resources are lower. Contrarily, their isotopic signature during the ice-free period, as in our case, agrees with a food resource of a pelagic origin. As state these authors, it is interesting to note that it implies a multi-chain omnivory, thus allowing zooplankton to maintain relatively high abundance in winter months.

Theory predicts that ecosystems' capability to be resilient to perturbations increases with biodiversity, in such a way that a higher occurrence of pathways in the food web increase the buffering capability. The comparison made by MARKAGER ET AL. (1999) between freshwater and marine ecosystems is a good example of this. These authors propose the idea that grazing exerted by zooplankton in high-latitude lakes is less effective than in polar oceans because the former contain grazers with a narrow array of particle-capturing mechanisms (i.e., they lack tunicates and choanoflagellates). Several studies have recognised the negative influence of the food web complexity on the occurrence of trophic cascades (POLIS ET AL. 2000 and literature herein). The food web typology of Lake Limnopolar seems to match these ideas. Nutritional versatility is an important strategy for survival in extreme systems and *B.poppei* is known to practice omnivory in other Antarctic lakes (BUTLER ET AL. 2005). Omnivory is favoured when high spatial and/or temporal variability occurs in food web components (THOMPSON ET AL. 2007). The omnivory phenomenon has been the subject of theoretical investigation, and it has been stated to be either stabilising or destabilising depending on background conditions (VANDERMEER 2006). In this way, it appears that its stabilising role based on the food web structure alone cannot be asserted. In any case, the capability of *B.poppei* to buffer phytoplankton dynamics is suggested in our experiments (see Experiment III in Chapter 6). Going back to the comparison

made by MARKAGER ET AL. (1999), here we can introduce the idea of a phenotypic rather than a genotypic diversity as adaptation to environmental uncertainty.

We have to face the question why Byers lakes show a less complex food web structure than that observed for instance in neighbouring locations, such as Signy Island (BUTLER ET AL. 2000) or King George (POCIECHA AND DUMONT 2008). For instance, an overwhelmingly dominance of *B.poppei* and the virtual absence of rotifers are both common trends in the Byers lakes. Several hypotheses can be put forward. Apparently there is no methodological bias to explain the scarcity of rotifers. The sampling method we used (a 30 µm net) seems adequate to retain them. We failed to discover if *B.poppei* controls rotifers populations by predation, or if it is the result of the overlap of niches that befall in a competitive exclusion. At times, predation exerted by copepods has been found to be the main factor reducing rotifers populations (DEVETTER 1998, CÉLIA ET AL 1999), even in the case of *Boeckella* species (GREEN ET AL 1999, COUCH ET AL 2001). Cladocerans are also scarce in Byers. Apparently large *Daphnia* and *Boeckella* species have a low food niche overlap (MODENUTTI ET AL. 2003). An alternative hypothesis, supported by both experimental and field observations, is that cladocerans (*Daphnia* spp.) are less UV-tolerant than copepods or rotifers (LEECH ET AL. 2000 and 2005, KESSLER ET AL. 2008). Byers lakes are greatly exposed to UV radiation, particularly during ice-free periods. A high photoreactivation potential seems to be a common trend in the red-coloured species of *Boeckella* (ZAGARESE ET AL 1997). In *B.poppei*, it has been particularly found that UV tolerance relates to the accumulation of mycosporine-like amino acids (ROCCO ET AL. 2002). Alternative explanations rely instead on nutritional constrains. There is the notion that the phosphorus requirement in cladocerans is around 5-fold higher if compared to copepods, which responds to a higher RNA content in the former (STEMBERGER AND MILLER 1998). On the other hand, the absence of cladocerans in terms of energetic economy can be explained as PRICE AND PAFFENHÖFER propose (1985). Based on direct observations, these authors explain how copepods, unlike cladocerans, reduce energy expense by limiting the feeding motions precisely when preys are detected. In this sense, filter-feeding cladocerans like *Daphnia* are known to suppress the whole microbial food web by overstressed predation (JÜRGENS ET AL. 1994). In unproductive systems such as the Byers lakes, the latter likely constitutes unfavourable behaviour. What most Byers lakes have in common with those from the Amery oasis is this unproductive character, with Chl-a levels usually below 1 µg L^{-1}. In contrast, the lakes from Signy Island usually have a higher trophic status (JONES ET AL. 1993). Some authors suggest that *B. poppei* lives close to the limit of tolerance on the Amery oasis, which would explain the dwarfism and the poor fecundity of the individuals observed there

(LAYBOURN-PARRY ET AL. 2001). The lakes from Hope Bay possibly represent a transitional state between Signy Island and Byers or the Amery oasis. Interestingly in the former, *B.poppei* dominates in oligotrophic waters, whereas it is totally absent in the nutrient-rich ones (IZAGUIRRE ET AL. 2003).

All the previously presented ideas are perhaps an oversimplified explanation because they are only reasoned in terms of species competition. Thus, it is possible that historical factors also account for this low metazoan diversity. A study based on a sediment core collected in Lake Limnopolar proposes that community shifts over the last ~2000 years respond mostly to the tephral inputs caused by catchment washing (AGIUS 2006). A tephrochronology performed in Midge Lake specifically dates these events at 1,340±100 yr BP (HODGSON ET AL. 1998). AGIUS suggests that the sequential changes observed in the sediment record reflect changes in the biota recovered from these episodic events. The interesting idea he introduced is that it involves an stressor that is harsh enough to allow the coexistence of more than a few zooplankton species. By accepting this hypothesis, the presence only of *B.gaini* and *B.poppei* would respond to a modern colonisation in an ecosystem far from the steady equilibrium. In paleontological records obtained in the lakes from James Ross Island (BJÖRCK ET AL. 1996), *B.gaini* has been shown to be more susceptible to high mineral loadings than to changes in temperature, which would support this idea. One possible weakness in this hypothesis is that it does not explain the occurrence of analogous metazoan assemblages at similar latitudes which are not affected by tephra inputs. In any case, these biogeographic considerations are beyond the scope of this thesis.

With regards to this strong dominance of copepods, other ideas can be introduced by observing the food webs structure of Patagonian lakes. In this region, there are lakes in which fishes have been introduced, thus resulting in different metazoan community structures (REISSIG ET AL. 2006). In the lakes undergoing this alteration, and dominated by *Boeckella* and *Parabroteas*, species richness diminished and rotifers increased its abundandes if compared to undisturbed lakes. Along with the disappearance of *Daphnia*, the zooplankton size spectrum tends to be narrower. In this case, it is generally observed that size-selective predators' activity favours small-bodied zooplankton via the suppression of competitors or intermediate predators. With the Patagonian lakes, these shifts in the zooplankton community structure cascaded to lower trophic levels in such a way that fishless lakes show a more heterogeneous phytoplankton community, while cyanobacteria dominate in lakes with introduced fishes.

There is a body of evidence indicating that quick ecological responses to environmental changes are taking place in Antarctica (CONVEY ET AL. 2002; QUAYLE ET AL. 2002). Longer productive periods with less restricting abiotic conditions will likely increase the relevance of such biotic interactions. The water from snow melting dramatically increases stream discharge, resulting in an increases nutrient transport from these channels to lakes. As far as other aspects are concerned, increasing temperatures may exhibit indirect effects that broadly transmit through systems. For instance, a shift in the carbon balance is expected because temperature regulates the photosynthesis/respiration ratio in such way that the most favourable temperature for photosynthesis is lower than that for respiration. Thereby, warming is expected to accelerate the carbon output caused by a shift in this respiration/photosynthesis balance. Based on this hypothetical case, pelagic bacteria's organic matter requirements that exceed primary production will increase even more. Furthermore, aquatic mosses are postulated to have lower photosynthesis rates compared to vascular macrophytes, which probably responds to the lower Rubisco activity (PROCTOR 1981). The direct consequence is that the increase in the respiration/photosynthesis ratios of these organisms would be even higher as temperature rises.

Our attempt to assay the impacts of simulated climate change (i.e., increase of predators and/or nutrient fertilisation) demonstrates that some ecological process and energetic flows could be less constricted in these extreme environments which are usually envisioned. A greater top-down control exerted by zooplankton may occur with warming not only because of an increment in populations, but also due to higher grazing rates. For instance, in a study recently carried out by GAEDKE ET AL. (2010) in the Baltic Sea region (with zooplankton mainly consisting of copepods), which combined both experimentation and mathematical modelling, the authors found that plankton trophic interactions overruled direct climate effects by a grazer-mediated inverse temperature effect. Therefore, the initial increase of phytoplankton abundances because of warming was counteracted by subsequently enhanced grazing, which finally produced reduced algal biomass. Our findings are of much interest in a climatic change scenario since warming is likely to intensify biotic interactions and biogeochemical cycles (DORAN ET AL 2002).

Conclusions

1. This thesis contributes to increase knowledge on the ecological functioning of freshwater ecosystems on Byers Peninsula, and therefore of the maritime Antarctica. We provide the soundest limnological report peformed to date in this region. Our findings show that the site is a unique location in Antarctica which deserves special attention. Water bodies at the site comprise a range of morphological, chemical and trophic conditions, and occur in a variety of different landscape units.

2. Most lakes show a thermal regime that varies between cold and temperate thereimictic, which agrees with that observed in other lakes of the region. This pattern is characterised by circulation only during summer. Lakes show a similar seasonal cycle to dimictic lakes, but without summer stratification.

3. The meteorological variation observed among the different years results in marked differences in the timing and duration of the summer ice-free period. As a result, the physical and chemical conditions changed notably. This demonstrates that ice dynamics is especially sensitive and very much subject to variations. It is for this reason that further attention has to be paid to the potential disagreement between the changes observed locally and those predicted by forecast models due to these year-to-year variations.

4. In Lake Limnopolar in particular, we show how wind and radiation regulate the heat exchange through the air-water boundary, thus affecting mixing patterns. The shear produced by wind over the lake surface extends through the water column, causing an excess water motion of the normal flow originating from the lake inlets. The greater losses of sensible heat (Qs) in the lake when ice-free conditions occur with the combination of relatively light winds and low solar radiation. Nonetheless, we observed certain thermal inertia in the lake, which causes delays in water column temperature changes in response to these atmospheric forces. In contrast, density-driven currents dominate when the lake is ice-covered. The main heat source at this time is penetrating solar energy, which produces a density-stratified water column.

5. Water bodies from Byers are low-mineralised and can be catalogued as the sodium type. They are characterised by a slightly acidic pH and low alkalinities. Our results indicate a major influence of rock/soils weathering in lakes' mineralisation. However, the Gibbs diagram suggests that waters are not totally in equilibrium with the surrounding catchment. For instance, the higher sodium content relating to calcium is higher than expected by rock weathering, suggesting a certain sea spray influence. It is for this reason that a major variation among lakes occurs along the inland-coastal gradient.

6. We propose that other differences in water mineralisation are related with catchment geology differences. For instance, lakes with lower silica concentrations are settled on the Cerro Negro Formation, where basalts are mainly composed of tuffaceous breccias with a low silica content (tephra). Yet there are also differences in the relative proportions of major cations depending on the lakes' location, which, responds to a sequential preponderance of less developed soils with the proximity of the glacier front.

7. We demonstrate the occurrence of natural eutrophication in the lakes, in such a way that they vary from ultra-oligotrophic to eutrophic conditions. In this sense, there are two major areas where lakes distribute. 1) A central plateau, lined with medium-depth and shallow lakes. These lakes have small watersheds and are ultra-oligotrophic. 2) The coastal area, which embraces the north, south and western beaches. Coastal lagoons commonly show nutrient concentrations and bigger watersheds. They experience nutrient input from surrounding fauna and are more exposed to sea spray. Among the lakes from the plateau, differences in trophic status are mainly due to their morphometric characteristics, to the extent that the internal inputs of nutrients are favoured in the shallower ones.

8. Bacterial abundances in lakes varied vastly from 0.5 to 6.5×10^6 cell mL^{-1}, and were dependent on the trophic status. These abundances were at any rate relatively high in oligotrophic waters. In the later, higher abundances occurred frequently at the onset or after of the ice thaw. In deeper lakes, higher numbers were usually recorded in sub-superficial layers. In Lake Limnopolar in particular, virus-like particles (VLP) showed greater abundances also in deep layers, with both absolute numbers (6.93×10^6 to 13.7×10^6 VLP mL^{-1}) and the Viruses:Bacteria ratios (3.47 to 7.29) correlating furthermore with copepods densities.

9. Our study demonstrates how ice cover affects the phytoplankton community structure. In general, autotrophic picoplankters were found in low densities in surface waters under open conditions (<1,000 cells mL^{-1}). Nonetheless, their contribution to total phytoplankton when Lake Limnopolar is ice-covered is occasionally greater at deep layers, in a range of 3.3-4.8 µg C L^{-1} and accounting for more than 50% of total phytoplankton. Contrarily during the ice-free period, chrysophytes and diatoms largely dominate, thus determining the phytoplankton succession pattern and reaching values of around 20 µg C L^{-1}.

10. The trophic differences among lakes also shape the structure of the phytoplankton community. An increase in nutrients favours a progressive dominance of chlorophytes over other algal groups (chrysophytes and/or diatoms). Moreover, a shift in the size structure is observed, which is reflected in the relative contribution of the smaller autotrophs. Thus, there is a trend towards reduced relative abundances of autotrophic picoplankters with an increase in trophic status, in such way that they show negatively and positively significant relationships with total phosphorus and the N/P molar ratio, respectively.

11. Byers lakes exhibit microbial-dominated communities. They support plankton in truncated pelagic food webs, at which the copepod *Boeckella poppei* is the dominant and capstone predator. However, small rotifers occur in very low numbers. The fairy shrimp *Branchinecta gaini* occurs near the bottom of some lakes, with a minor impact on the pelagic compartment. A small number of flagellates species (both colourless and plastidic forms), small amoebae and ciliates, mainly *Balanion planktonicum*, compose the protozoan community. Among flagellates, plastidic forms generally dominate.

12. The isotopic fractionation of carbon in Lake Limnopolar suggests that *B.poppei* interacts poorly with the benthos and exploits principally pelagic resources. However, it is possible that this copepod changes its feeding behaviour depending on environmental conditions. The vertical distribution of copepods during some periods in the lake, showing individuals mainly retreated to deep layers, supports this idea and suggests a nektobenthic feeding mode. In contrast, the results indicate that *B.gaini* benefit from benthic resources, thus indicating a niche segregation between both species. Otherwise, the highly depleted values of seston from the catchment indicate that it is mainly composed of autotrophic biomass which, in our opinion, could subsidise the lake's heterotrophic metabolism.

13. The use of migration traps has revealed some vertical movements of *B.poppei*. However, their vertical distribution is always heterogeneous, as previously mentioned, particularly during the periods when they abound, when most of the population is found close to the bottom and coincides with maximal picoplankton abundance. Despite the higher light depletion below the ice and the possible relaxation of circadian rhythms, some copepods ages reveal a diel pattern of migration. These movements could determine a translocation of nutrients driven by copepods from deep layers to the nutrient-poor surface.

14. We have experimentally demonstrated the potential existence of a trophic cascade in the microbial pelagic food web of Lake Limnopolar. Accordingly, copepods predation diminishes the density of protozoa populations; consequently, bacterivory declines and picoplankton biomass accumulates. Experiments indicate that this could be channelized through predation on ciliates (*Balanion planctonicum*), whose abundance declines markedly in response to increases in copepod densities. This top-down control also produces a shift of the bacterial community size structure.

15. The grazing experiments performed with microspheres additionally sustain this idea. Thus, the preferred particle-size ingested by adult copepods match the size of the euplanktonic ciliates occurring in the lake, and is also quite similar to most nanoplanktonic phytoplankton. In contrast, smaller nanoflagellates mainly benefit for immature copepods. The seasonal uncoupling observed between copepods and protozoan abundances in Lake Limnopolar agree with these observations.

16. The top-down effects of copepods also act as a shaping force of phytoplankton assemblage by causing a redistribution of the relative abundances of algal groups. Variations of taxa-specific carotenoids amounts indicate that chlorophytes, but not diatoms and chrysophytes, are favoured when copepods abound, which is related with the edibility of the species involved. We suggest that both larger and pico-sized chlorophytes are beyond the adult copepods' prey range.

17. Copepods-driven nutrients recirculation may promote fertilisation effects to the extent that it seems high enough to over-compensate for some groups the losses caused by grazing. In particular, this may occur via nitrogen turnover by both the dissolution of faecal pellets and the "sloppy feeding" mechanism. The latter is an inefficient feeding form that depends on prey edibility so that detritus increases when the prey is larger in relation to the predator. We estimate that this nutrient recycling could be significant in these lakes given their general oligotrophic conditions.

18. The environmental conditions of Byers Peninsula are suitable for the extensive growth of mat-building microorganisms. These microbial mats distribute in wet areas. We have studied three communities types as being representative of the diversity observed at the site. Two occur in the central plateau (soil and pond mats) and the other on beaches' streams (stream mats), and depending on location, they vary in terms of their taxonomic composition, structure and physiological activity.

19. The two microbial mats located inland (soil and pond mats) are dominated by cyanobacteria throughout their vertical profiles, whereas diatoms are more abundant in the stream mat. The areal photosynthetic (mainly oxygenic) rates in all three mats were in the range of 2.7-4.2 μg C cm^{-2} h^{-1}, and were near 2-fold higher in the stream mat. Conversely, N_2 fixation only occurs in soil and pond mats, but mainly in the former. The areal assimilation rates of combined nitrogen (mostly ammonium) are higher in stream mats, which furthermore show a more equilibrated stoichiometry for both nitrogen and phosphorus.

20. Despite the taxonomic differences, the three studied mats show the same basic structure. They have a thicker, unstructured and inactive surface layer, largely composed of empty cyanobacterial sheaths and diatom shells, and a subsurface stratum assembled by a competent photosynthetic biomass with a more equilibrated stoichiometric composition. The profiles with microelectrodes show maximum photosynthetic rates occurring at these basal layers. The higher exopolymeric substances content observed in the upper stratums, which is furthermore higher in the mats from the plateau, may also respond to this nutrient unbalance.

21. We experimentally tested part of the Tilman's resource ratio hypothesis in a microbial mat to reveal that competitive abilities may also regulate species distribution in a physically constrained environment depending on nutrients equilibriums. In this sense, we found that higher phosphorus availability favours the growth of non-heterocystous cyanobacteria in relation to diatoms. The results also indicate that assimilative and loss processes are closely balanced, which translate into no mat build-up despite nutrient enrichment. However, enhanced photosynthetic activity is observed if inorganic nutrients requirements are not limited.

22. A considerable level of spatial heterogeneity may also occur within the stream' environment, thus allowing the simultaneous occurrence of different phototrophic biofilms. Accordingly, we observed up to 5 biofilms occurring in a waterfall which formed a canyon downstream. Each biofilm exhibits a different assemblage of chlorophytes, cyanobacteria and diatoms, thus conforming different communities. Here chlorophytes progress with faster flows, whereas cyanobacteria appear in a wide range of environmental conditions, including those exposed to greater drought stress.

23. The exopolimeric substances content, stoichiometry and pigment composition of these biofilms reveal a distinct nutritional status, which is likely motivated by different exposures to drought stress. Areal photosynthetic rates also vary in this sense, with higher carbon uptakes (2.4-3.4 μg C cm^{-2} h^{-1}) occurring in those biofilms with higher moisture.

24. Our outcomes permit us to conclude that biotic interactions may play a key role in structuring the freshwater communities studied despite environmental harshness. The results of nutrient enrichment experiments led us to suspect that a shift in regional nutrients dynamics might alter some functional aspects of these microbial communities. Also important could be the top-down interactions controlling the planktonic community structure, mainly in a framework of increasing temperatures that dwindle the strength of climatic restrictions in polar areas.

Conclusiones

1. La presente tesis contribuye a incremetar el conocimiento del funcionamineto ecológico de los sistemas acuáticos epicontinentales de la Península Byers y por extensión de la región de la Antártida marítima. Esta tesis constituye el estudio limnológico más profundo llevado a cabo en esta región en particular, redundando por ello en la peculiaridad que esta posee desde un punto de vista ecológico. Los cuerpos de agua estudiados son representativos de la zona y presentan distintas características morfológicas, químicas y estado trófico.

2. La mayoría de los lagos estudiados muestran un régimen térmico que varía entre monomíctico polar y templado, lo que coincide con lo observado en otros lagos de la región marítima del continente. Este patrón se caracteriza por la existencia de circulación sólo durante el verano austral. En este sentido, estos lagos muestran un ciclo estacional similar a los lagos dimicticos, aunque sin mostrar estratificación estival.

3. La variación meteorológica inter-anual observada de la zona implica la existencia de marcados cambios en la dinámica de la cobertura de hielo. Como consecuencia de esto, las condiciones físicas y químicas de los lagos se ven afectadas de forma notable. Nuestro estudio demuestra la alta sensibilidad y grado de variabilidad que pueden presentar estas dinámicas. Es por ello que se debe prestar una especial atención a las potenciales discrepancias existentes entre los cambios observados a nivel local y las predichas en los modelos de cambio global.

4. En el Lago Limnopolar en particular, se ha estudiado la regulación del intercambio de calor a través de la superficie mediante la perdida de calor sensible (*Qs*), lo que condiciona los patrones de mezcla. La abrasión producida por el viento sobre la superficie del lago se extiende a través de la columna de agua, aumentando así la turbulencia, la cual puede exceder a la observada por el flujo de los tributarios. Las mayores pérdidas de calor sensible se producen en ausencia de hielo, con la combinación sostenida de vientos relativamente ligeros y una radiación solar baja, lo que podría deberse en parte a la batimetría del lago. Sin embargo, se observa una cierta inercia térmica en la masa de agua, la cual produce demoras en los cambios de temperatura de la columna de agua en respuesta a estas fuerzas atmosféricas. Esto contrasta con el predominio de corrientes baroclínicas observado durante los periodos en los que la superficie del lago esta congelada, las cuales responden a un calentamiento diferencial de distintos estratos de la columna de agua.

5. Los cuerpos de agua de Byers presentan una baja mineralización, pudiendo ser catalogados como de tipo sódico. Se caracterizan por presentar un pH ligeramente ácido y alcalinidades bajas. Los resultados indican una importante influencia de la geología de la zona en la determinación de dicha mineralización. En cualquier caso, el diagrama de Gibbs sugiere que las aguas no se encuentran totalmente en equilibrio con las características de la cuenca. Así, el contenido de sodio en relación al calcio es mayor del esperado, lo que indica la existencia de aportes marinos a través de aerosoles. Este hecho explicaría que las mayores variaciones se observen en base al eje litoral-interior.

6. Otras diferencias observadas en la mineralización de los lagos podrían estar relacionadas con variaciones en la geología de la zona. Así, los lagos que muestran las concentraciones más bajas de silicatos se encuentran ubicados en la Formación Cerro Negro, donde los basaltos se componen principalmente de tefras volcánicas con bajo contenido en sílice. Por otra parte, algunas diferencias en las proporciones relativas de los cationes principales, podría responder a una disminución secuencial de la evolución de los suelos conforme aumenta la proximidad del frente del glaciar.

7. Se ha demostrado la existencia de procesos de eutrofización naturales que afectan a los sistemas acuáticos estudiados, de tal modo que estos varian de ultra-oligotróficos a eutróficos. Es posible distinguir en este sentido dos áreas geográficas en Byers: 1) Un altiplano caracterizado por la presencia de lagos someros o escasamente profundos. Estos lagos son generalmente oligotróficos y presentan cuencas relativamente pequeñas. 2) La zona costera, que abarca las playas del norte, sur y oeste. Las lagunas en esta zona son someras, suelen presentar concentraciones más altas de nutrientes y cuencas de captación más extensas. Estas lagunas experimentan un aporte de nutrientes proveniente de la fauna circundante y están más expuestos a los aerosoles marinos. Entre los lagos del altiplano, las diferencias en el estado trófico responden a sus características morfológicas, de tal modo que una mayor carga interna de nutrientes se ve favorecida en los más someros.

8. Las densidades de bacterioplancton en los lagos variaron en torno a 0,5 y 6,5x10^6 células mL^{-1}, estando estas subordinadas a su estado trófico. En cualquier caso, estas densidades fueron relativamente elevadas incluso en los lagos más oligotróficos. En estos casos, las mayores abundancias se observaron normalmente durante el inicio el deshielo o justo en el periodo posterior. En los lagos más profundos, las mayores densidades se observaron generalmente en estratos sub-superficiales. En el Lago Limnopolar en particular, el virioplancton tuvo también una mayor presencia en capas profundas, tanto en términos absolutos (6,93-13,7x10^6 VLP mL^{-1}) como relativos a la densidad de bacterias (3.47-7.29), estando además estas densidades correlacionadas con la abundancia de copépodos.

9. Nuestro estudio demuestra el efecto que la dinámica de la cubierta de hielo tiene sobre la estructura de la comunidad fitoplanctónica. En general, en aguas superficiales y en ausencia de hielo, el picoplancton autótrofo presentó bajas densidades (<1.000 células mL^{-1}). Su contribución fue, por el contrario, ocasionalmente elevada antes del deshielo, con valores de entre 3,3 y 4,8 µg C L^{-1}, llegando a representar entorno al 50% de la biomasa total de fitoplancton. Por el contrario, durante los períodos libres de hielo, las crisófitas y diatomeas determinaron en gran medida el grueso de la población, alcanzando valores máximos de en torno a 20 µg C L^{-1}.

10. El estado trófico de los lagos determina también la estructura de la comunidad fitoplanctónica. El aumento de nutrientes favorece un dominio progresivo de las clorófitas en relación a otros grupos de algas (crisófitas y/o diatomeas). Por otro lado, se observa también un cambio en la estructura de tamaños, reflejado en la variación de la contribución del picoplancton autótrofo a la biomasa algal total. Se observa así una tendencia hacia la reducción de la abundancia relativa del picoplancton conforme aumenta el estado trófico, de tal forma que se observan correlaciones significativas, positivas y negativas respectivamente, con el fósforo total y la relación molar N/P.

11. Los lagos estudiados presentan cadenas tróficas incompletas. El copépodo *Boeckella poppei* es la especie del zooplacton dominate, siendo además el depredador superior. Los rotíferos, por el contrario, están practicamente ausentes en estas comunidades. El anostráceo *Branchinecta gaini* aparece estrechamente ligado al bentos, generando un impacto mínimo sobre el plancton. La comunidad de protozoos está compuesta por unas pocas especies de flagelados, tanto heterótrofos como autótrofos, amebas y ciliados, principalmente *Balanion planktonicum*. Las formas autótrofas son generalmente las dominantes entre los flagelados.

12. El fraccionamiento isotópico del carbono en el Lago Limnopolar indica que *B.poppei* interactúa poco con el bentos y explota principalmente recursos pelágicos. Sin embargo, es posible que este copépodo cambie su modo de alimentación dependiendo de las condiciones ambientales. Así, el hacinamiento de individuos en capas profundas observado durante algunos períodos en el lago sugiere un comportamiento nectobentónico. En el caso de *B.gaini*, el fraccionamiento corrobora el carácter netamente bentónico de esta especie, lo que demuestra una clara segregación de nichos entre ambas especies. Por otro lado, el fraccionamiento indica también un predominio de biomasa autótrofa en el seston alóctono, la cual podría contribuir en parte a sostener el metabolismo heterótrofo del lago.

13. El uso de trampas de migración ha puesto de manifiesto la capacidad de *B.poppei* para llevar a cabo movimientos verticales en la columna de agua. El confinamiento de individuos cerca del fondo coincide además con las máximas abundancias de picoplancton en estas capas profundas. A pesar de la notable extinción de la luz debida a la presencia del hielo y a la posible relajación de los ritmos circadianos, algunos estadios juveniles mostraron un patrón diario de migración. Estos movimientos podrían determinar una translocación de nutrientes desde las capas profundas a las más superficiales del lago.

14. Se ha demostrado experimentalmente la potencial existencia de una cascada trófica en el plancton del Lago Limnopolar. En particular se observa como la depredación llevada a cabo por los copépodos ejerce un fuerte control sobre las poblaciones de protozoos, disminuyendo en consecuencia la bacterivoría, lo que a su vez permite que se acumule biomasa del picoplancton. Los experimentos indican que, bajo un dominio de estadios adultos de *B.poppei*, esto ocurre principalmente a través de la depredación ejercida sobre los ciliados (*Balanion planctonicum*), cuya poblaciones se reducen de forma notable en respuesta al aumento de la densidad de copépodos.

15. Los experimentos de depredación llevados a cabo con microesferas apoyan esta idea. Así, el tamaño de partícula mayoritariamente ingerida por los copépodos adultos coincide en buena medida con el tamaño de *Balanion planctonicum*, y es también similar a parte del nanofitoplancton presente en el lago. Por el contrario, los estadios inmaduros muestran una mayor predilección por tamaños similares a los de los nanoflagelados de pequeño tamaño. El desacoplamiento temporal observado entre las poblaciones de copépodos y protozoos en el Lago Limnopolar sostiene estas observaciones experimentales.

16. El control top-down ejercido por los copépodos tiene su efecto también sobre la estructura de la comunidad fitoplanctónica, causando una redistribución de las abundancias relativas de los distintos grupos algales. Así, la variación de los carotenos taxón-específicos indica un efecto positivo del zooplancton sobre las clorófitas y negativo sobre las diatomeas o algas crisófitas, lo que puede responder a la susceptibilidad de cada presa a ser ingerida. En este sentido, tanto las clorófitas picoplanctónicas como las de mayor tamaño parecen estar fuera del espectro de presas de los copépodos adultos.

17. La recirculación de nutrientes promovida por los copépodos ejerce un efecto de fertilización positivo. Esta recirculación parece lo suficientemente importante como para compensar las pérdidas de bioamasa causadas por la depredación sobre parte de la población del fitoplancton. Lo que se observa en particular es una regeneración del nitrógeno, la cual puede deberse tanto a la disolución de partículas fecales como al "*sloppy feeding*", el cual consiste en un mecanismo de alimentación ineficiente que deriva en una mayor producción de detritus conforme aumenta el tamaño relativo de la presa. Debido a la general escasez de nutrientes, estos mecanismos de reciclaje de nutrientes podrían ser relativamente importantes en este tipo de lagos.

18. Las condiciones ambientales de la península Byers favorecen el desarrollo de tapetes microbianos. Estas comunidades se encuentran distribuidas por distintas zonas húmedas de la región. En particular se han estudiado tres tipos de tapetes microbianos, los cuales configuran en gran medida la diversidad observada en la zona. Dos de ellos se distribuyen mayoritariamente en el altiplano (*soil* y *pond*) y el tercero es más abundante en los sistemas lóticos de la zona costera (*stream*). Dependiendo de su ubicación, estos tapetes varían en su composición taxonómica, estructura y actividad fisiológica.

19. Los dos tapetes microbianos del altiplano (*soil* y *pond*) muestran una presencia mayoritaria de cianobacterias filamentosas a través de todo su perfil. Por el contrario, las diatomeas pennadas son más abundantes en el tapete de la zona costera (*stream*). Las tasas fotosintéticas en los tres tapetes fueron principalmente oxigénicas y oscilaron entre 2,7 y 4,2 μg C cm^{-2} h^{-1}, siendo más altas en el tapete *stream* de la zona costera. Por el contrario, sólo se observó fijación de N_2 en los tapetes del altiplano, particularmente en el tapete *soil*. Las tasas de asimilación de nitrógeno combinado (mayoritariamente en forma de amonio) fueron más elevadas en el tapete de la zona costera, el cual mostró además una composición estequiométrica más equilibrada.

20. Los tres tipos de tapetes microbianos estudiados muestran una estructura básica similar a pesar de las diferencias taxonómicas existente entre ellos. Se componen de un estrato superior formado principalmente por biomasa inactiva, en gran parte compuesto de vainas vacías de cianobacterias y frústulos de diatomeas; y una capa inferior compuesta por biomasa fotosintéticamente competente, con una composición estequiométrica más equilibrada. Los perfiles realizados con microelectrodos muestran igualmente una mayor actividad fotosintética en estas capas sub-superficiales. El mayor contenido de sustancias exopoliméricas en los estratos superiores, en particular en los tapetes del altiplano, podría responder también a este desequilibrio nutricional.

21. En uno de los tapetes microbianos estudiados se ha comprobado experimentalmente parte de la aplicabilidad del modelo de Tilman de utilización diferencial de recursos, con la intención de valorar en que medida la competencia inter-específica puede regular la estructura de estas comunidades incluso en un entorno con elevado control físico. Los resultados muestran que una mayor disponibilidad de fósforo favorece el crecimiento de las cianobacterias frente a las diatomeas. Los resultados sugieren también que estas comunidades se encuentran en un estado de equilibrio estacionario, lo que se traduce en la no acreción del tapete a pesar de la adicción de nutrientes. En cualquier caso, sí se observa un estímulo de la actividad fotosintética cuando no existe una limitación por nutrientes.

22. Se ha constatado la existencia de un nivel considerable de heterogeneidad espacial dentro de los sistemas lóticos de la región, lo que permite la presencia simultánea de biofilms fotosintéticos con distintas características estructurales. En particular, se reconocieron un total de 5 biofilms en un salto de agua presente en uno de los drenajes de un lago. La dominancia relativa de clorófitas, cianobacterias y diatomeas varió en cada uno de ellos. Los biofilms dominados por clorófitas se restringen a las zonas con mayor flujo de agua, mientras que aquellos dominados por cianobacterias se desarrollan en una mayor variedad de condiciones, incluyendo aquellas sometidas a mayor estrés hídrico.

23. El contenido de sustancias exopolimericas, estequiometría y composición pigmentária de estos biofilms revela un desigual estado nutricional, hecho probablemente motivado por una diferente exposición al estrés hídrico. La actividad fotosintética en los distintos biofilms varió en este sentido, observándose las tasas más altas de asimilación de carbono (2,4-3,4 μg C cm^{-2} h^{-1}) en los biofilms menos expuestos a este factor de estrés.

24. Nuestros resultados permiten concluir que las interacciones bióticas descritas aquí pueden jugar un papel clave en la estructuración de las comunidades estudiadas a pesar del fuerte control físico al que están sometidas. Lo observado sugiere que un cambio regional en el ciclo de nutrientes podría alterar alguno de los aspectos funcionales de dichas comunidades. Las interacciones resultado de las relaciones tróficas entre organismos podrían tener también una mayor relevancia, en particular en un escenario de aumento de las temperaturas, el cual provocaría una reducción de las restricciones climáticas en las zonas polares.

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